EVALUATION OF REPRODUCTIVE BIOLOGY AND MORPHO-ANATOMICAL VARIATIONS IN *Plumbago spp.*

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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COLLEGE OF HORTICULTURE
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KERALA, INDIA

2000

DECLARATION

I hereby declare that this thesis entitled **"Evaluation of reproductive biology and morpho-anatomical variations in *Plumbago spp.*"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associate-ship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Evaluation of reproductive biology and morpho-anatomical variations in *Plumbago spp.*"** is a record of research work done independently by **Subha, K.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associate-ship to her.

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Introduction

INTRODUCTION

Plants are vital for the existence of life in the universe. Other than synthesizing food, they also provide chemicals necessary for human health. Indian historic books record various uses of herbal medicines since vedic times. Following the advent of modern medicine, herbal medicine suffered a set back. However, the advances in phytochemistry and identification of plant compounds effective against certain diseases, have renewed the interest in herbal medicine.

More than 60 percent of Indian population still depends on ayurveda for the treatment of common diseases (Nair *et al.*, 1992). Plants and plant products are main source of various ayurvedic preparations. Nearly 2000 plant species are used in ancient and modern system of medicines (Chatterjee and Nandi, 1983).

Medicinal plants are the foremost in the list of endangered species, their cultivation being restricted to small pockets of tribal areas. But these plants face high demand after the modern studies, which revealed them as potent diagnostic sources with relatively less side effects. So as to satisfy the consumer demand of such a limited source, in many cases, samples are adulterated. *Plumbago* is one of such plants, which is valued for their roots that possess medicinal quality.

Plumbago spp belong to the family Plumbaginaceae of the order Plumbaginales. It comes under the series Heteromerae (Bentham and Hooker,

1884). Hutchinson (1973) and Engler (1973) reported that *Plumbago* comes under order plumbaginales. The genus *Plumbago* includes 20 species. Three species *Plumbago rosea* L., *Plumbago zeylanica* L. and *Plumbago capensis* T. are recorded from India based on flower color.

The red flowered type *Plumbago rosea* is the accepted source of drug in Kerala. The species is reported to be indigenous to Sikkim and Khasi hills. Its cultivation is always associated with anthropogenic localities in both north and south India indicating its use as a tribal medicine.

The roots form the officinal part of the plant that enters into the composition of preparations like Citrakasavam, Dasamoolaristam etc. The active principle in *Plumbago* is plumbagin (2-methyl 5 hydroxy 1,4 naphtoquinone). It is obtained as golden yellow needle shaped crystals (Chopra *et al.*, 1958). It is known to have antifungal (Ito *et al.*, 1995), antimicrobial (Gonclaves *et al.*,1972), abortifacient (Goel *et al.*, 1987), antifertility (Chowdary *et al.*, 1982), anticoagulant (Santhakumari *et al.*, 1978), antiviral (Singh *et al.*, 1983) and insecticidal (Rao and Gujar, 1995) properties. It is also effective in treatment of liver disorder (Gujar, 1990) and common warts (Pillai *et al.*, 1981). It is a powerful irritant as well as antiseptic agent (Krishnaswamy and Purushothaman, 1980). It is also reported to cause chromosomal aberrations.

The average annual requirement of *Plumbago* roots is 400t. Roots appear in the market as cylindrical pieces of varying length. In many cases, samples are adulterated with plant organs of similar morphology. There is at present no scientific control at any stage of collection of roots, and it passes through so many hands that indiscriminate adulteration or substitution is possible.

In the light of above facts the present investigation was undertaken to evaluate and correlate the morphological and anatomical features existing in different species of *Plumbago* as well as to compare the reproductive biology of different species of *Plumbago.*

Review of literature

REVIEW OF LITERATURE

Plumbaginaceae is the only family of order Plumbaginales. It contains 19 genera and 775 species. Members of this family are mainly herbs and shrubs (Trease and Evans, 1985).

There are several related genera viz., *Plumbago, Plumbagella, Armeria, Limonium, Goniolimon* and *Acantholimon* reported in the family Plumbaginaceae (Darlington and Wylic, 1961).

Under genera *Plumbago* there are three species viz., *Plumbago rosea* Linn., *Plumbago zeylanica* Linn., *Plumbago capensis* Thumb recorded from India of which red flowered *Plumbago rosea* is the accepted source of drug in Kerala (Sivarajan and Balachandran, 1994).

Subha (1990) compared the effect of different levels of spacing and planting materials on yield and quality of *Plumbago rosea*. The results indicate that plumbagin content of root in this species varied from 0.16 % - 0.17 %. Different spacing levels did not influence the plumbagin content.

The possibility of successfully cultivating *Plumbago* as an intercrop in young coconut plantation, adopting different methods of planting such as ridge and furrow, flat bed, mound and pit followed mound was observed by Menon (1995). No significant difference was observed in root yield under open and shaded condition. But higher shoot weight was observed under

shaded condition. Even tough crude plumbagin was higher under shade condition, there was no marked difference in the purified plumbagin content.

Roots of *Plumbago* form an esteemed remedy for skin diseases. It is also a digestive stimulant and aids digestion. Plumbagin is the main active ingredient in *Plumbago.* CSIR (1969) reported the chemical nature of plumbagin as a naphthoquinone (2 methyl, 5 hydroxy, 1,4 naphthoquinone).

Britton (1983) reported that plumbagin is basically a naphthalene derivative, 1,4 naphthoquinone with methyl and hydroxy substituents. 1,4 naphthoquinones are most widespread and important as pigments.

Kitanov and Pashanov (1994) reported that yellow pigment plumbagin in *Plumbago* species exhibit various pharmacological and insecticidal activities. The studies on quantitative aspects revealed that plumbagin content is 3.06 % in flowers during peak period of anthesis (September), 0.04 - 0.08 % in young stems and leaves combined, 1.35 - 1.65 % in older stems, 2.07 % in young root, and 1.53 % in perennial root of *Plumbago europea.* It was also reported that the flowering shoots form alternative plumbagin source to root.

Gupta and Verma (1995) isolated a compound 2,2 methyl 5-hydroxy 6 acetyl chromene from *Plumbago zeylanica.* It was the first time that this chemical was isolated from a natural source.

2.1. Morphological characters

Iyer and Kolammal (1960) described the floral biology of *Plumbago zeylanica* and *P. rosea.* They found that in *P. zeylanica* bracts are considerably larger than bracteoles where as they are nearly equal in size in *P. rosea.*

Plumbago capensis, native of South Africa is a shrub with oblong or oblong spatulate leaves and pale blue flowers. This species is grown in gardens of India as an ornamental plant (CSIR, 1969).

Glandular hairs are present on the leaves, branches and fruits of *Plumbago zeylanica.* These hairs secrete a sticky substance (Dutta, 1970).

Scott (1994) reported 'Monott' a new cultivar of *Plumbago auriculata* which is a compact densely growing shrub possessing glossy green leaves. It flowers profusely over the warmer months producing groups of rich blue flowers which have larger petals and darker color than *P. auriculata.*

Dajoz (1990) reported pollen dimorphism in *Viola diversifolia.* In all populations studied 3, 4, 5, and 6 apertured pollen grains were observed.

Nyo (1994) studied the taxonomy of family Asclepidaceae, one of the largest family in flora of Myanmar with 36 genera and 87 species. He found that grouping of genera could be done based on the nature of stamens, features of anther lobes and pollen grains.

Lokesha *et al.* (1996) reported existence of four categories of morphologically distinguishable flowers within same inflorescence of *Caesalpinia pulcherrima*. The four types varied with respect of their stylar length. Flowers with long, medium, short and rudimentary styles were found of which long styled type was functionally hermaphrodite and was produced towards basal region of inflorescence.

2.2. Anatomical characterization

Iyer and Kolammal (1960) studied root anatomy of *Plumbago rosea* and *Plumbago zeylanica*. They reported the presence of starch grains in the cortex and medullary rays of *Plumbago zeylanica* roots and presence of coloring contents in the cortex and medullary rays of *P. rosea* roots with which species could be differentiated.

Carlquist and Boggs (1996) studied wood anatomy of Plumbaginaceae. They found that certain features like simple perforation plates, small pits on lateral vessel walls and paratracheal axial parenchyma seen in some species of this family suggests a close relationship with polygonaceae.

Menon (1999) studied root anatomy in *Plumbago rosea* and *Plumbago zeylanica* and reported the presence of sclerenchyma in the cortex of *P. zeylanica* roots which is an indication of drought tolerance of the crop.

A tendency for phylogenetic decrease in length of the vessel elements in angiosperms corresponding to advancement was reported by Bailey and Tupper (1918) and Cheadle (1943).

Iyer and Kolammal (1966) reported that *Piper longum* could be distinguished from adulterants by its anatomical characters like thick walled cells in cortex, narrower phloem, and darker colored cork tissue.

Ayensu (1970) reported the presence of varied number of vascular bundles in different species of *Discorea*. Bundle number was eight in *D.cayensis* where it was 12 in *D. rotundata*.

De Padua (1978) found that the large sac like structures in the section of root is a key factor of identification of *Symphytum officinale*.

Servettaz *et al*. (1980) studied histology and development of cortex of *Peumus boldus* and reported the cortex thickness to be 0.25 mm in very thin branches. It reached upto 6 mm in well developed branches.

Singh and Srivastava (1980) did morphological and anatomical studies in stem of *Costus speciosus* collected from different geographical regions of country and found that plants occurring wild in different regions of country are one and same form and morphological variations are only climatic adaptation.

Lal and Khan (1981) studied the stem anatomy of *Portulaca quadrifolia* and *Portulaca oleraceae*. They reported that

Portulaca oleraceae had double the number of vascular bundles compared to *Portulaca quadrifolia*.

Anatomical studies on the stem of *Caesalpinia sappan* revealed presence of calcium oxalate crystals in the cortical cells with interspersed stone cells in it. In addition a well developed xylem and scattered group of fibers were also present (Mehrotra and Sharma, 1983).

Shome *et al.* (1984) studied pharmacognosy of vegetative parts of *Artemisia scoparia* and reported that presence of casparian strips in the endodermis, resin canal and 4 - 7 celled glandular hairs can be considered as diagnostic features of the drug.

Singh *et al.* (1986) studied stem anatomy of *Alternanthera sessilis* and reported the presence of 10 - 14 fine grooves, two opposite lines of hairs and collenchyma under each ridge in the T.S of this species.

Heubl *et al.* (1988) did anatomical investigation in root of *Echincea spp* and found that sclerenchymatous stone cells are of great importance in species identification.

Anatomical studies in the roots of *Nilgirianthus* showed the presence of pigmented crystoliths in the cortex and pith region, and thick walled oval or elongated stone cells in the cortex. These are reported to be diagnostic feature of the drug (Shantha *et al.*, 1988).

The root anatomical characters-bordered pit vessels and lack of fibers can be considered as identifying markers of *Taraxacum sp.* (Langer, 1990).

Zheng *et al.* (1991) reported the existence of wide variations in thickness of pith and cork region in different species of genus *Plantago* with which different species could be distinguished.

Chauhan and Dayal (1992) studied wood anatomy of Indian species of *Michelia* and found that features like size and shape of vessels, presence or absence of oil cells in rays and presence or absence of spiral in vessels are of diagnostic value in identification.

Shylaja and Manilal (1992) identified sclerenchymatous cells in the pericycle region and crystalline inclusions as the key identifying factors in *Cinnamomum.*

Subrata *et al.* (1993) studied the stem anatomy of *Gymnosporia montana* and observed the presence of broken sclerenchymatous pericycle fibres, very large isolated prismatic squarish and rhomboidal calcium oxalate crystals and dark colored cell depositions as characteristic features of the species.

Dey and Das (1995) reported the presence of bundles of sclerenchymatous fibers in *Pedalium murex* roots.

Stem anatomy of *Polygala* genus showed the presence of a single row of chlorenchyma under stem epidermis (Kim *et al.*, 1996).

Vadukkoot (1996) studied xylem vessel characters in four species of *Ocimum* and reported that *O. basilicum* and *O. canum* having circular and pitted vessels are more evolved than the other two species *O. tenuifolium* and *O. grattisimum* having angular vessels.

The stone cells and fibers are found to be specific markers of crude Chinese drugs and products containing them (Lhao *et al.*, 1997).

Certain parameters like presence of short vessels with simple perforation plates at transverse ends and simple pits with alternative arrangement are advanced evolutionary characters (Pupuma and Bhat, 1997).

2.3. Reproductive Biology

Baker (1966) reported the presence of papillate and nonpapillate type of stigmas in several species of Plumbaginaceae.

Dulberger (1975) reported that existence of stigma and pollen dimorphism reflects incompactability mechanism in family plumbaginaceae. Inhibition of pollen growth was found to occur on the stigmatic surface.

Frankel and Galun (1977) observed the existence of heteromorphic incompatibility in *Plumbago*, *Ceratostigma* and *Limonium* of family plumbaginaceae.

Bahadur (1978) noticed distyly as common feature of several members of family plumbaginaceae.

Escher *et al.* (1988) found that *Plumbago indica* requires a day length of nine hours for flowering.

Huang *et al.* (1990) studied the organization of embryosac and egg of *Plumbago zeylanica*. They reported that egg of *P. zeylanica* has combined synergids and gamete functions.

Huang and Russell (1993) reported that embryosac of *P. zeylanica* consists of 2 to 5 cells including an egg cell, a central cell and 0 to 1 antipodal cell depending on the degeneration of lateral and chalazal nuclei during megagametogenesis.

Nihayati *et al.* (1995) studied the effect of water application at different intervals on flower number, quality of flowers and flower freshness. It was found that increasing intervals resulted in lowest flower number, smallest flower diameter and lower flower freshness.

Sodmergen *et al.* (1995) studied male gametophyte development in *Plumbago zeylanica* and explained cell localization and cell determination in early generative cell of *Plumbago zeylanica*.

Microgametogenesis and three dimensional organization in *Plumbago zeylanica* was studied by Russell *et al.* (1996).

Cao-Yajuan *et al.* (1997) isolated viable egg of *Plumbago zeylanica* and found that eggs retain viability for 24 hours.

Southward *et al.* (1997) noticed the existence dimorphic sperms in *Plumbago zeylanica.* The plastid rich sperms fused with egg and sperm with fewer plastids fused with the central cell.

Arya (1999) reported abundant adhesion pollengrains on papillate stigma in *Plumbago zeylanica* unlike *Plumbago rosea* as one reason for satisfactory seed set in this species.

Krisek and Semeniuk (1972) observed that a low temperature of 16-13^0 C is required for flower initiation in *Limonium.*

Semeniuk and Krisek (1972) reported that long days and cool night temperature increased the percentage of flowering in *Limonium* cultivars.

A temperature of >13^0 C with 16h photoperiod advanced onset of flowering in *Limonium sinensis* by six weeks. It also results in increase in the inflorescence width (Lopez *et al.*, 1996).

Mitra (1970) reported colporoidate type of pollen in *Capparis* species. Pollen is with obscure surface pattern in this genus.

Chaturvedi and Gupta (1982) studied pollen morphology in *Capparis* species. He reported that ultra surface pattern coupled with endocolpium characters can be used for species identification.

Jayachandran and Vijayagopal (1979) studied floral biology of ginger *Zingiber officinale* and reported that blooming occured in acropetal sucession. Pollen grains are spherical and pollen sterility ranged upto an average pf 76 %.

Mercy *et al.* (1979) reported existence of four distinct stages in the anthesis of lemon grass. Large number of spikelets opens in a short span of time and this indicates the possibility of self-pollination in this crop.

Studies on floral biology of *Emblica officinalis* revealed the presence of monad type of pollen grains that are spheroidal and non coherent. Anther dehisces in the evening time and pollination is aided by wind (Reddi and Bai, 1979).

The anthesis in maximum number of florets occurred during early morning hours in *Plantago ovata* while stigma maturity occurred both in morning and evening hours (Patel *et al.*, 1980).

Pollen morphology studies of *Papaver somniferum* revealed that they are characteristically 3 – zonocolpate. Exine surface was fundamentally spinate. It was found that the two cultivars 'Kentia' and 'Aphudi' could be distinguished by the presence of non spinate humps in 'Kentia' which is absent in 'Aphudi' (Sharma, 1980).

Mattason (1983) emphasized the significance of pollen derived exine oils in the interaction between pollen and stigma and successful pollination in *Armeria*.

Heslop - Harrison (1987) reported that elongation of pollen tube in the pistil tissue is promoted and guided by pistil derived nutrients in several plants.

Filippini *et al.* (1990) reported the presence of 3-colporoidate prolate pollen grain with reticulate exine and co-occurance of smooth branched warty, striated, non-branched trichomes as valuable diagnostic characters of commercial powered products of *Verbasum* flowers.

Floral biology studies on bael revealed 4 - 5 a.m. as peak period of anthesis and 7 - 8 a.m. as peak period of anther dehiscence (Singh, 1991).

Naik *et al.* (1996) studied floral biology of *Oenothera lamarkiana* and reported 9 - 10 a.m. as peak period of anthesis and 9 a.m. as peak period of anther dehiscence. Pollen diameter was 173.5 μ and stigma receptivity was 100 % on the day of anthesis.

Since the literature available on *Plumbago* and related species is meagre, relevant works on other crops like *Viola* were also reviewed.

In this background the present investigation will form a basis for future crop improvement works in *Plumbago*.

Materials and methods

MATERIALS AND METHODS

An evaluation of three species of *Plumbago* viz., *Plumbago rosea*, *Plumbago zeylanica* and *Plumbago capensis* based on phytographic and anatomical characters as well as reproductive biology was carried out at the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during the period from April 1997 to December 1999.

3.1. Morphological Evaluation

For each species the observations on the following morphological characters were recorded and a descriptor was prepared (Radford *et al.*, 1974).

a. General habit
b. Root type
c. Stem type
d. Internodal length
e. Leaf type
f. Leaf shape
g. Leaf size
h. Leaf arrangement
i. Leaf attachment
j. Leaf margin
k. Leaf apex
l. Leaf colour
m. Venation
n. Inflorescence character
o. Fruit and seed character

3.2. Anatomical characterization.

3.2.1. Stem Anatomy

Free hand transverse sections were taken from the first internode of the unflowered first primary branches of each species. Sections were then made permanent following the procedure described by Prasad and Krishnaprasad (1970). Sections were stained and counterstained for five minutes in one percent aqueous saffranine solution and washed in distilled water until excess stain was removed. They were dehydrated by passing through graded concentrations of alcohol. The sections were then counterstained with light green SF in clove oil (1:1) for two minutes, washed in clove oil, passed through xylene and mounted in canadabalsam.

3.2.2. Root Anatomy

Mature roots of three species of *Plumbago* were collected and free hand transverse sections were taken and made permanent following the procedure described by Prasad and Krishnaprasad (1970).

3.2.2.1. Study of Xylem Vessels

For studying features of xylem vessels, the internodal segments were macerated by treating with Jeffery's fluid (Prasad and Krishnaprasad, 1970). Jeffery's fluid was prepared by mixing equal volumes of 10 % chromic acid and 10 % nitric acid. The treatment time was five minutes. The macerated tissue was washed thoroughly in distilled water and stained with one percent

aqueous saffranine solution. Then it was mounted on clear slides and examined under microscope. The pattern of thickening as well as the length and breadth of xylem elements of each species were measured using ocular micrometer. For each species observations were taken from 50 vessels of each species.

3.3. Reproductive Biology

3.3.1. Time of flower opening

A preliminary trial was conducted by tagging ten mature but unopened inflorescence of each species. Inflorescences were observed at 2 a.m. and at 4 p.m. All the opened flowers were clipped off and inflorescences were again observed at 2 a.m. next day to see whether any flower opening was there between 4 p.m. and 2 a.m. After preliminary studies, inflorescences of uniform age facing different directions in five different plants of each species were labeled. The number of flowers opened in each inflorescence was recorded at bihourly intervals from 2 a.m. to 4 p.m. The counting was continued from the commencement till completion of flowering in an inflorescence. Percentage of flowers opened at bihourly intervals were estimated for determining peak time of flower opening on each day and peak period of anthesis in an inflorescence.

3.3.2. Time of anther dehiscence and stigma receptivity

The color and appearance of anthers were examined with hand lens at bihourly intervals in fully matured flower buds of each species to find out time

of anther dehiscence in a flower. The stigmatic surfaces was also observed for any change in colour or appearance in the same buds, at same intervals of time to find out stigma receptivity.

3.3.3. Pollen morphology and fertility

Pollen morphology of all the three species of *Plumbago* was studied taking pollen from fully opened buds. The morphological features like size and shape, sculpturing of exine were examined for each species. Pollen diameter was measured using ocular micrometer, after calibration. Mean size of pollen was computed. The sculpturing of exine was examined under microscope and classification was done following the procedure suggested by Moore and Webb (1978).

Fertility of pollen was assessed on the basis of stainability of pollengrain in acetocarmine - glycerine mixture. Pollengrains were collected from the mature buds and stained with a drop of acetocarmine - glycerine mixture on clean slide and kept aside for ten minutes. All the pollengrains that were well filled and stained were counted as fertile and others as sterile. Five fields of five different slides were prepared from each species, and were observed under microscope. The values were expressed as percentage.

3.3.4. Mode of pollination

This experiment was conducted with a view to find out the role played by pollinating agents. Twenty inflorescences of uniform age were tagged before the commencement of anthesis. Out of these, ten were covered with butter paper to encourage self pollination and the remaining ten left uncovered so as to favor open pollination. The extent of fruit set in each case were determined and expressed as percent.

The unweighted pair group method of cluster analysis using the arithmetic averages (Sneath and Sokal, 1973) was done taking into consideration 27 characters. The cluster analysis helped to produce hierarchial classification of entries based on the similarity matrix and distance matrix.

Results

RESULTS

4.1 Morphological evaluation

Evaluation of three different species *Plumbago rosea, Plumbago zeylanica, Plumbago capensis* in respect of morphological, anatomical and floral characters were undertaken during 1997-1999.

4.1.1 Vegetative characters

Observations on various morphological characters of root, stem and leaves of the three different species of *Plumbago* were recorded and the results are presented in Table1 and plate1.

Table 1. Morphological characters of three different species of *Plumbago*

Character	*P. rosea*	*P. zeylanica*	*P. capensis*
General habit	Shrub	Shrub	Shrub
Root type *	Fibrous	Fibrous	Fibrous
Stem type	Smooth type	Ridged with reddish striations along ridges starting from the node	Angular
Mean Internodal length (cm)	6.90	6.00	3.10
Leaf type	Simple	Simple	Simple
Leaf arrangement	Alternate- spiral	Alternate- spiral	Alternate-spiral
Leaf texture	Smooth	Smooth	Smooth
Venation	Reticulate	Reticulate	Reticulate
Leaf attachment	Petiolate	Petiolate	Sessile

Plate 1 Leaves of three different species of *Plumbago*

1. *P. rosea*
2. *P. zeylanica*
3. *P.capensis*

Plate 2 Inflorescence of three different species of *Plumbago*

1. *P. rosea*
2. *P. zeylanica*
3. *P.capensis*


1
2
3

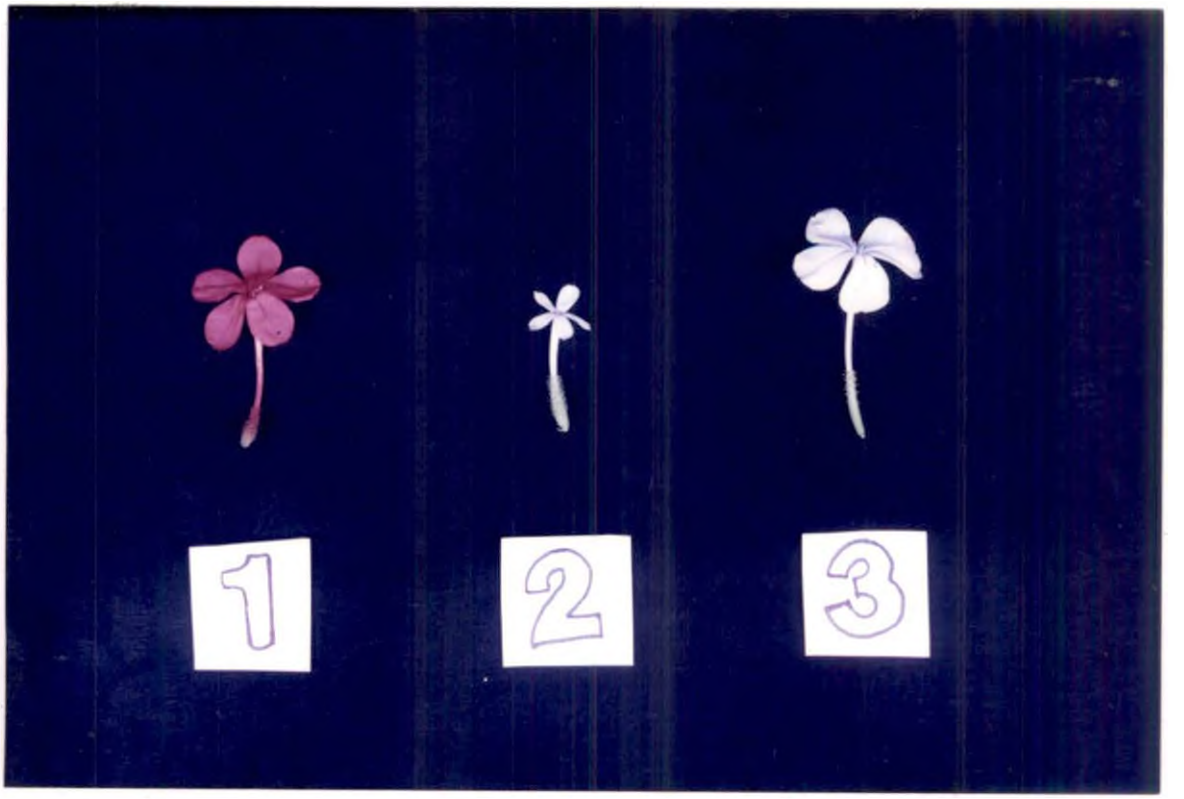

1
2
3

Plate 3 Flowers of three different species of *Plumbago*

1. *P. rosea*
2. *P. zeylanica*
4. *P.capensis*

Plate 4 Bracts of three different species of *Plumbago*

1. *P. rosea*
2. *P. zeylanica*
3. *P.capensis*


1
2
3

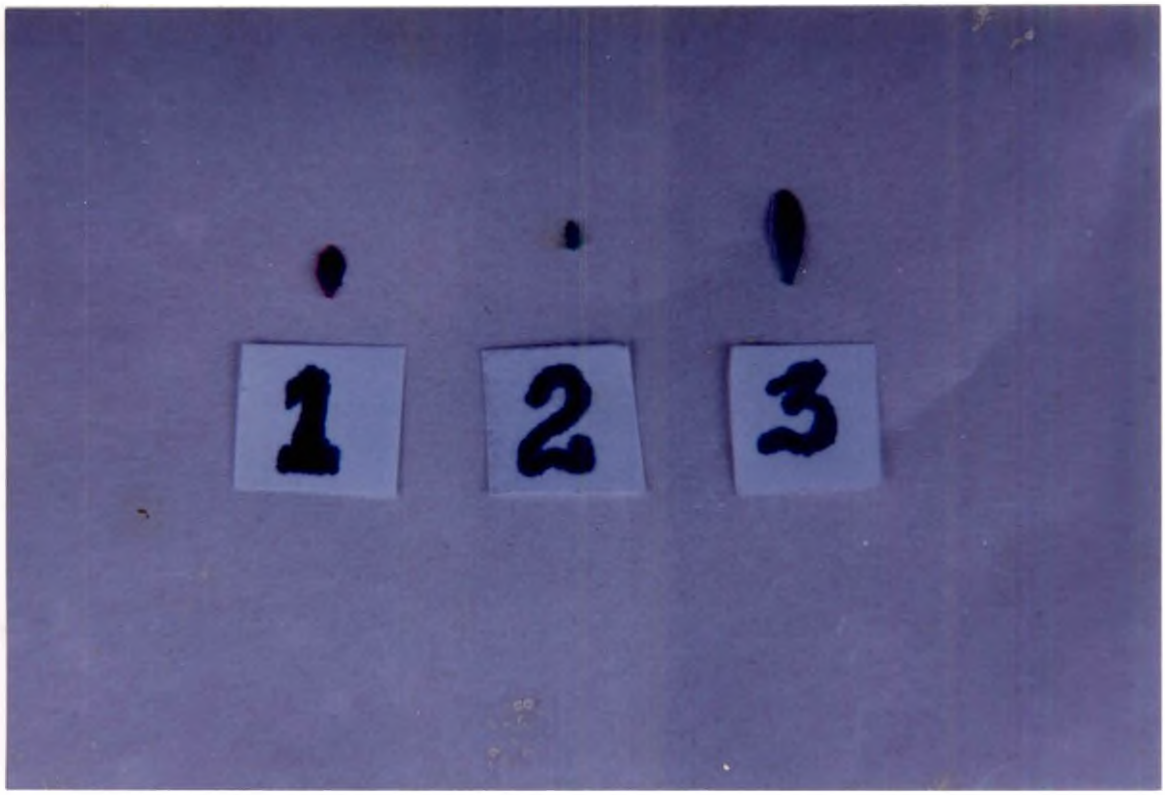

1
2
3

Plate 5 Bracteoles of three different species of *Plumbago*

1. *P. rosea*
2. *P. zeylanica*
3. *P.capensis*

Plate 6 Calyx of three different species of *Plumbago*

1. *P. rosea*
2. *P. zeylanica*
3. *P.capensis*


1
2
3


1
2
3

Plate 7 Corolla of three different species of *Plumbago*

1. *P. rosea*
2. *P. zeylanica*
3. *P.capensis*

Plate 8 Androecium and Gynoecium of three different species of *Plumbago*

1. *P. rosea*
2. *P. zeylanica*
3. *P.capensis*

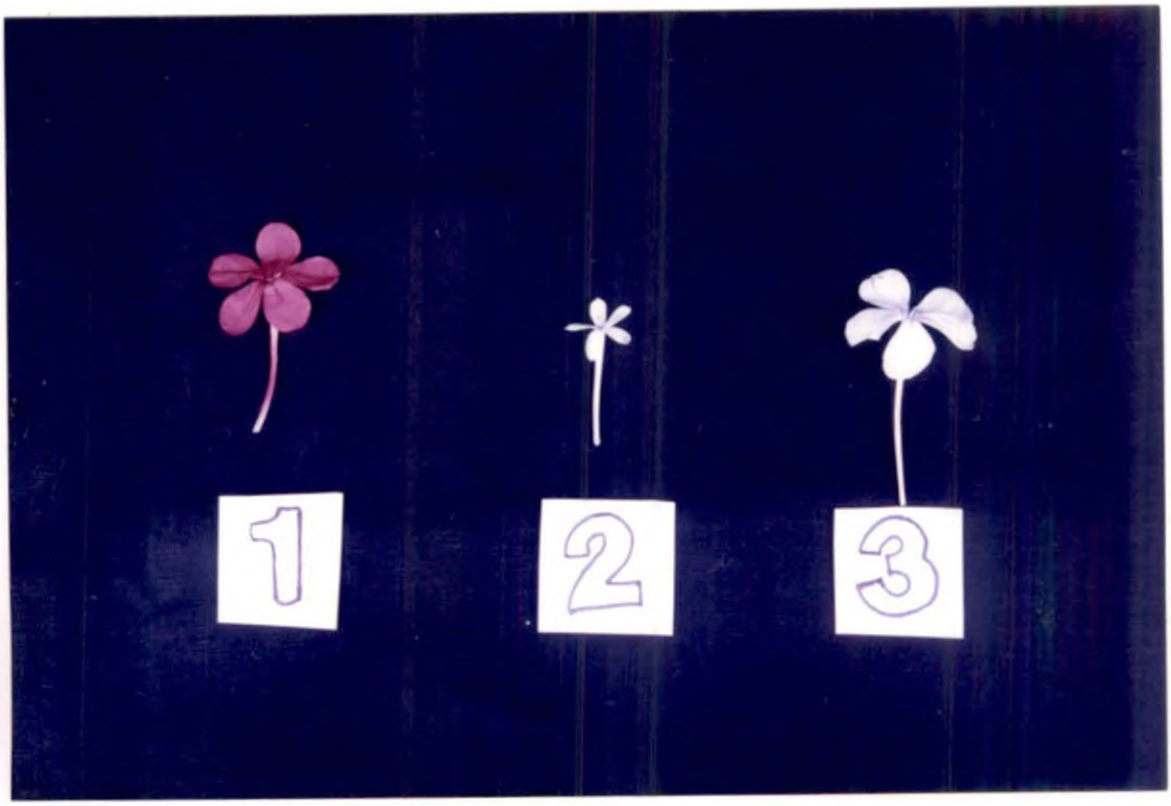

1
2
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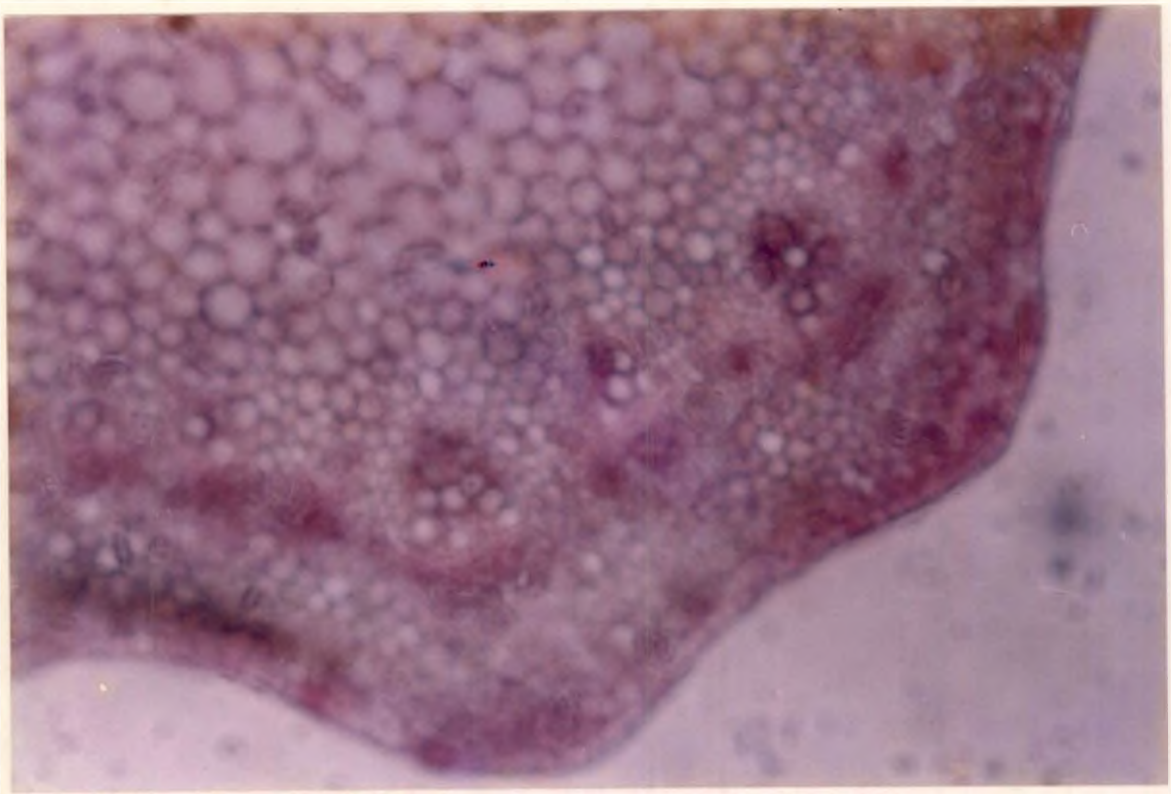
Plate 9 Stem anatomy of *Plumbago rosea* (X100)

Plate 10 Stem anatomy of *Plumbago zeylanica* (X100)



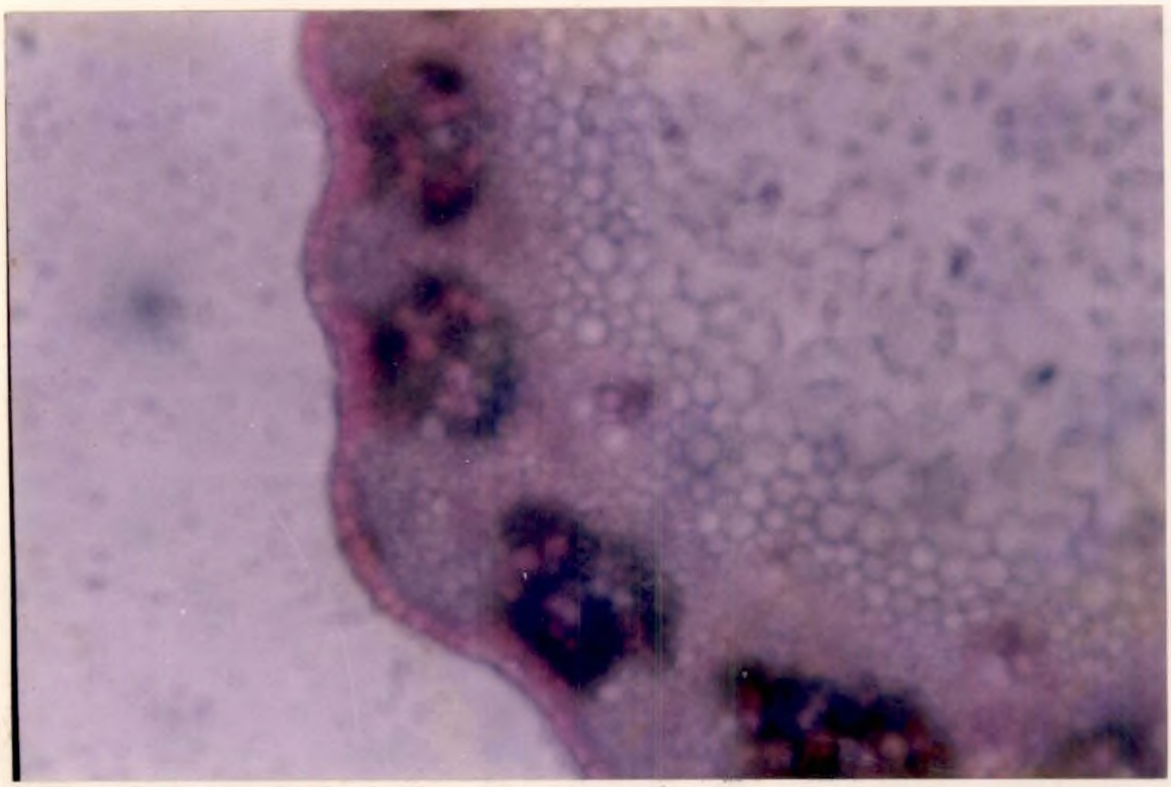


Plate 11 Stem anatomy of *Plumbago capensis* (X100)

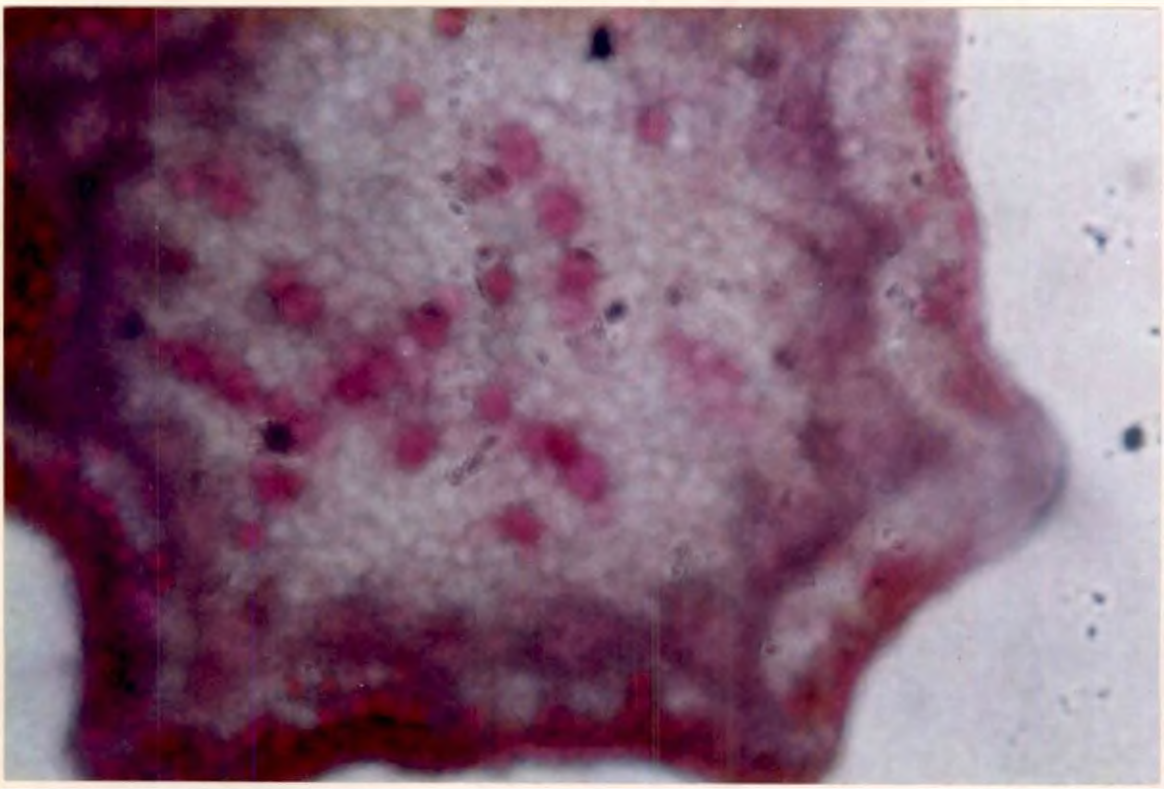
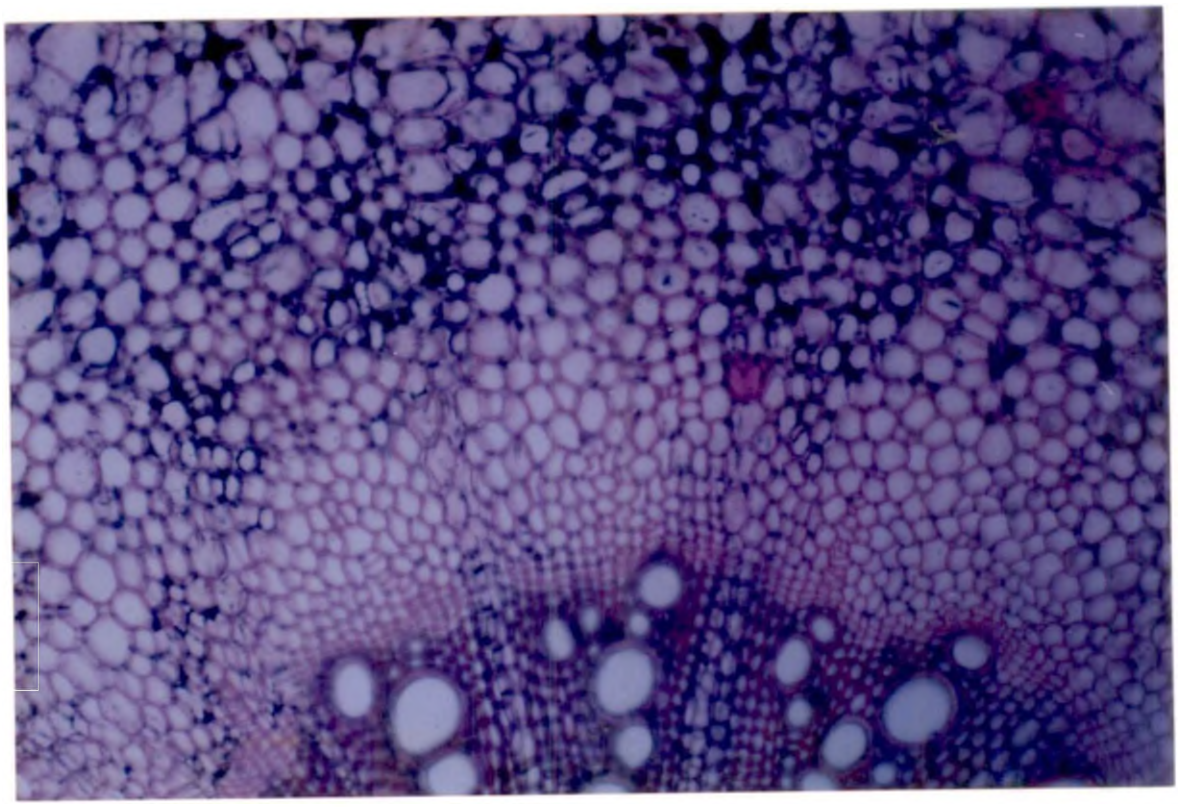


Plate 12 Root anatomy of *Plumbago rosea* (X100)

Plate 13 Root anatomy of *Plumbago zeylanica* (X100)



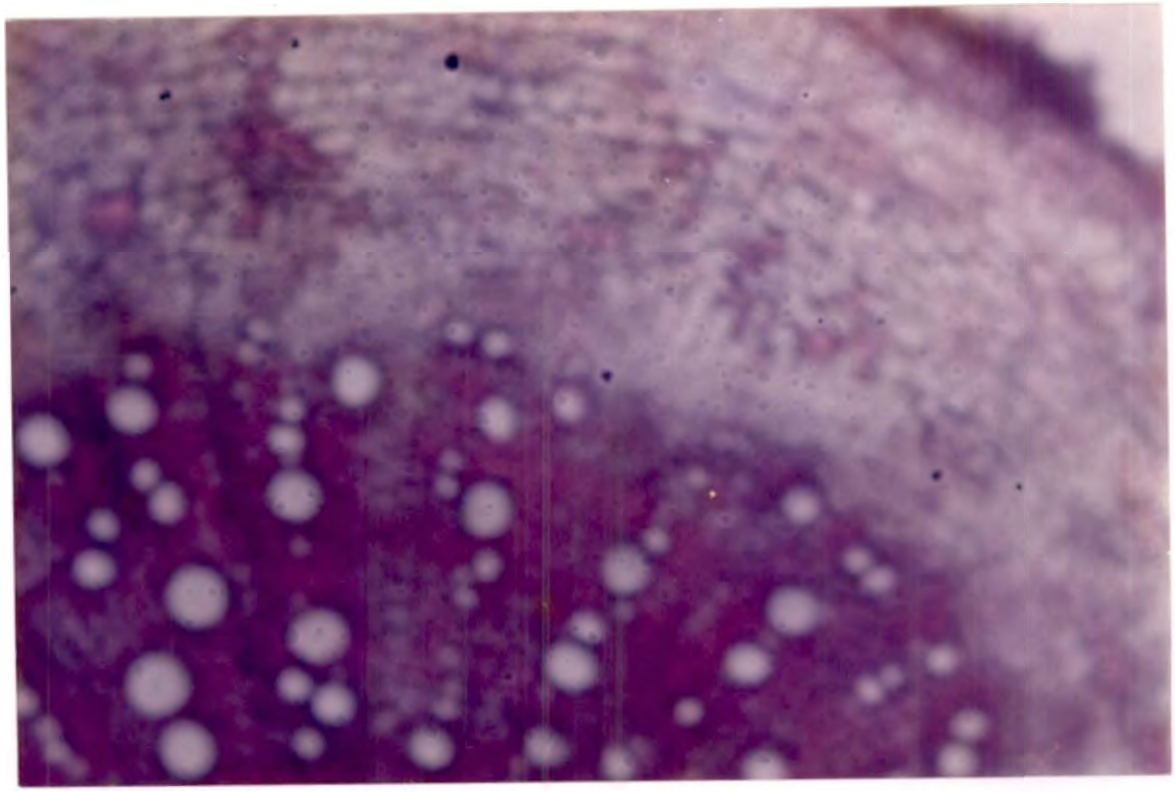
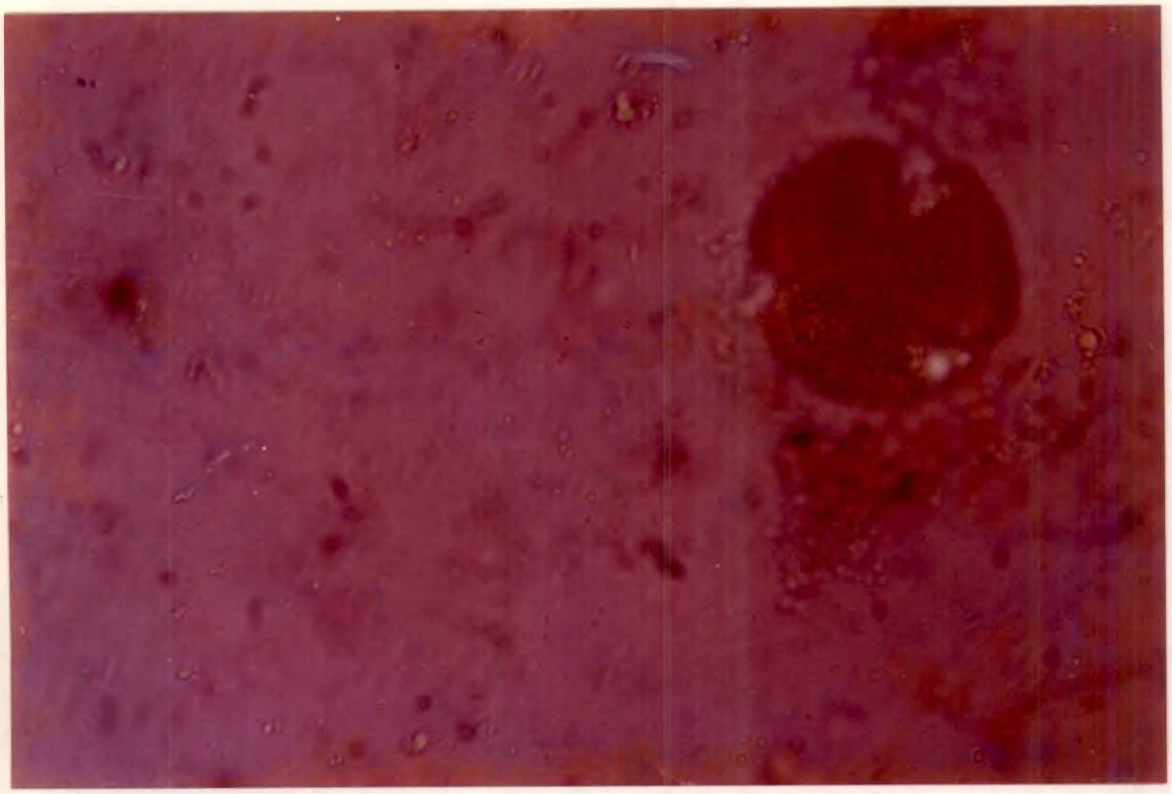


Plate 15 Pollen grains of *Plumbago rosea* (X400)

Plate 16 Pollen grains of *Plumbago zeylanica* (X400)



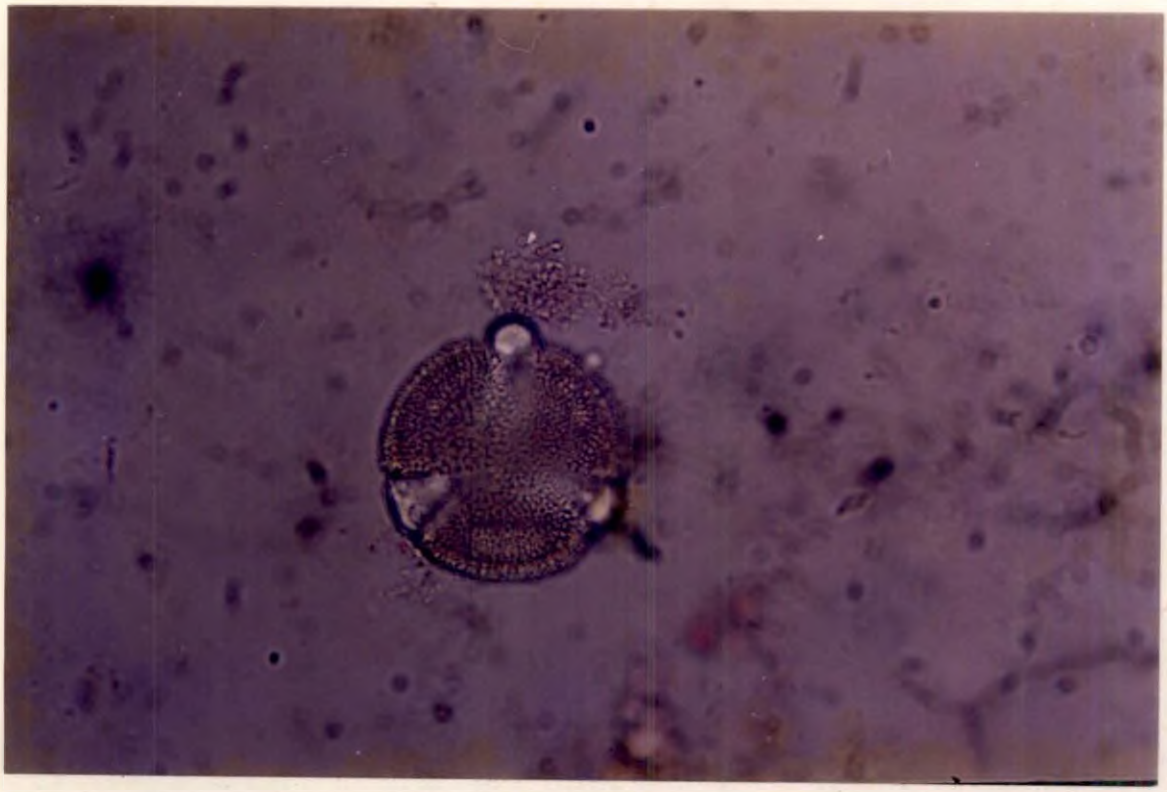
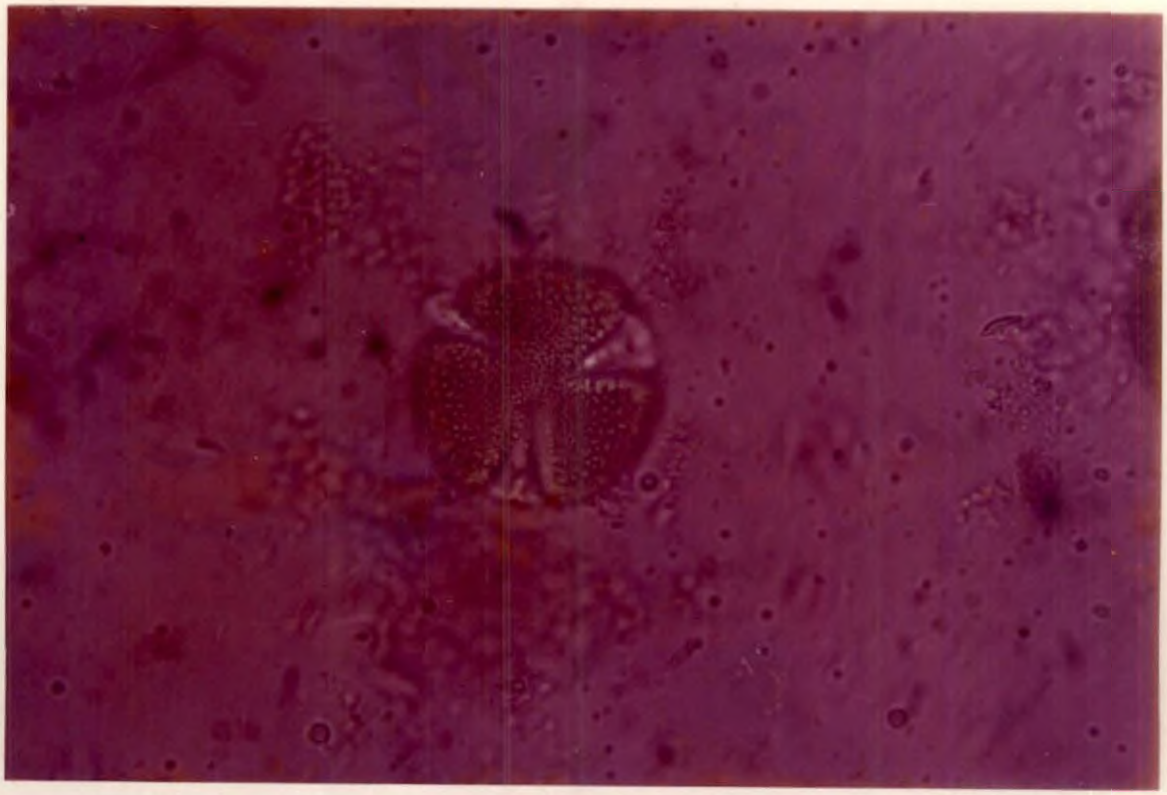


Plate 17 Pollen grains of *Plumbago capensis* (x400)



Leaf color	Dark green (petiole and midrib have reddish tinch)	Green	Light green
L:B ratio	2:1	2:1	3:1
Leaf shape	Ovate	Ovate	Spatulate
Leaf apex	Acute	Acute	Acute
Leaf margin	Entire	Entire	Entire
Stipules	Absent	Absent	Absent

(*Clonal progenies were observed)

From the above table variations can be observed with respect to internodal length, leaf color, leaf attachment, leaf size and leaf shape among the three different species.

4.1.2 Inflorescence characters

Inflorescence is a spike in all the three species of *Plumbago*. Characters of inflorescence were studied and the results are presented in Table 2 and plate 2.

Table 2. Inflorescence characters of different species of *Plumbago*

Character	*P. rosea*	*P. zeylanica*	*P. capensis*
Flowering	Seasonal	Seasonal	Continuous
Inflorescence type	Spike	Spike	Spike
Position of inflorescence	Terminal	Terminal / axillary	Terminal / axillary
Mean length of inflorescence (cm)	26.63	11.27	4.26
Color of peduncle	Dark green	Green	Light green

Mean number of flowers / inflorescence	32.40	26.10	12.90
Mean distance between flowers (cm)	0.87	0.47	0.32
Number of flowers / unit length of inflorescence	1.20	2.26	3.14
Mean time taken for completion of anthesis / inflorescece (days)	9	6	4

From the above table it is clear that the three different species of *Plumbago* differed with respect to flowering time, length of inflorescence, color of peduncle, number of flowers per inflorescence etc. *Plumbago capensis* was found to produce flowers round the year.

4.1.3 Floral characters

Floral characters of the three species of *Plumbago* viz. *P. rosea*, *P. zeylanica*, and *P. capensis* were observed and are presented in Table 3 and plates 3 to 8.

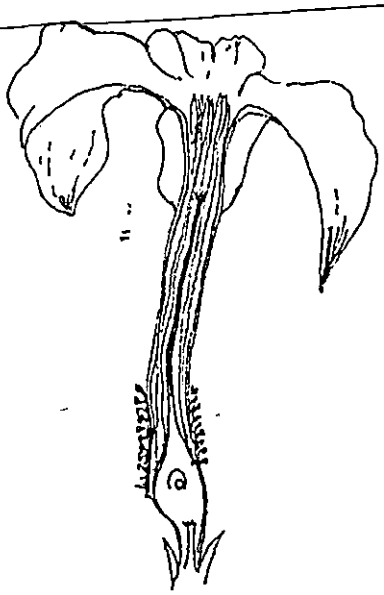
Table 3. Floral morphology of different species of *Plumbago*

Character	*P. rosea*	*P. zeylanica*	*P. capensis*
Sequence of flower opening	Acropetal	Acropetal	Acropetal
Flower colour	Pinkish red	White	Blue
Flower type	Sessile	Sessile	Sessile
Flower sex	Bisexual	Bisexual	Bisexual
Flower symmetry	Actinomorphic	Actinomorphic	Actinomorphic

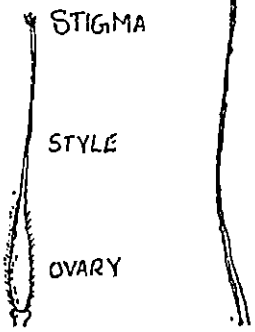
Bract			
Colour	Reddish green	Green	Green
Shape	Cordate	Cordate	Lanceolate
Mean size	0.50 cm x 0.27 cm	0.68 cm x 0.29 cm	0.84 cm x 0.19 cm
Bracteole			
Colour	Reddish green	Green	Green
Shape	Lanceolate	Cordate	Lanceolate
Mean size	0.39 cm x 0.19 cm	0.26 cm x 0.15 cm	0.49 cm x 0.18 cm
Number of braceteolates / flower	2	2	2
Calyx			
Mean size of calyx tube (cm)	0.85	1.07	1.15
Colour	Red	Green	Light green
Number of sepals	5	5	5
Nature of sepals	Gamosepalous, sticky and hairy	Gamosepalous, sticky and hairy	Gamosepalous, sticky and hairy
Corolla			
Colour	Pinkish red	White	Blue
Mean size of corolla tube (cm)	2.80	2.30	3.50
Mean size of limb (cm)	1.70	0.68	1.20
Androecium			
Anther attachment	Dorsifixed	Dorsifixed	Dorsifixed
Mean length of stamen (cm)	3.06	2.32	3.50

Stamen structural type	Filantherous	Filantherous	Filantherous
Colour of anther	Red	Purple	Blue
Colour of filament	Pink	White	Blue
Staminal type	Epipetalous	Epipetalous	Epipetalous
Pollen type	Monad	Monad	Monad
Pollen dehiscence	Longitudinal	Longitudinal	Longitudinal
Colour of pollen	Creamy white	White	Creamy yellow
Gynoecium			
Ovary	Superior, unilocular	Superior, unilocular	Superior, unilocular
Placentation	Basal	Basal	Basal
Type of style	Fimbricate	Terete	Terete
Colour of style	White	White	White
Mean length of style (cm)	2.30	2.27	2.72
Type of stigma	Pentafid	Pentafid	Pentafid
Fruit type	No fruit set	Dry dehiscent fruit enclosed in persistent calyx	No fruit set
Seed type	_	Black, oval seeds with one end pointed	_

Table 3 shows that marked differences exist among the three species of *Plumbago* for various floral characters. In all the three species, each flower is subtended by a bract and two bracteoles. The colour, size and shape of bracts and bracteoles were characteristic for each of the three species. Floral formula and floral diagram are given in Fig. 1 to 3.

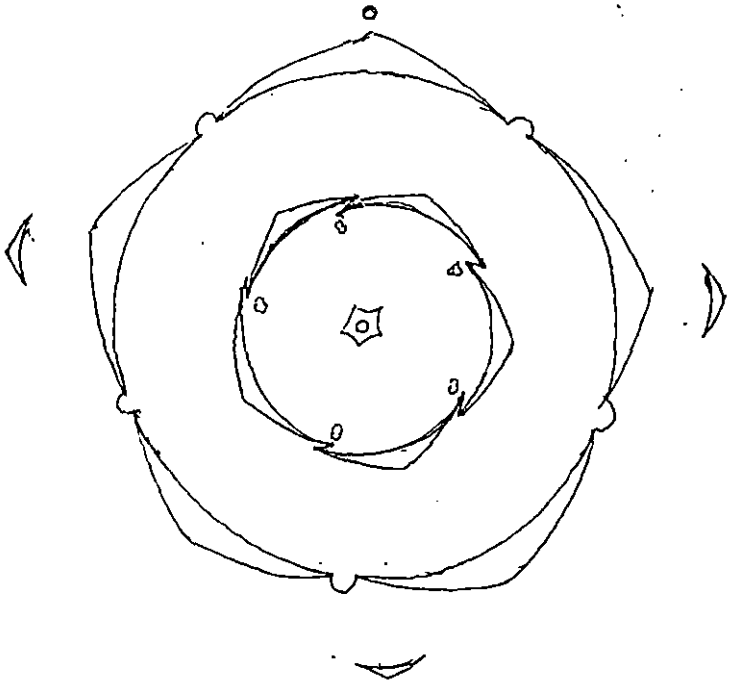


L.S OF FLOWER



STAMEN

C.S. OF OVARY



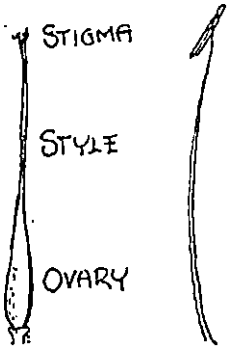
FLORAL DIAGRAM

$$\varphi \oplus K_{(5)} C_{(5)} A_5 \underline{G}_1$$

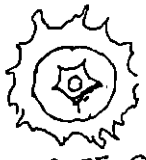
FLORAL FORMULA

Fig.1 Floral morphology of *Plumbago rosea*

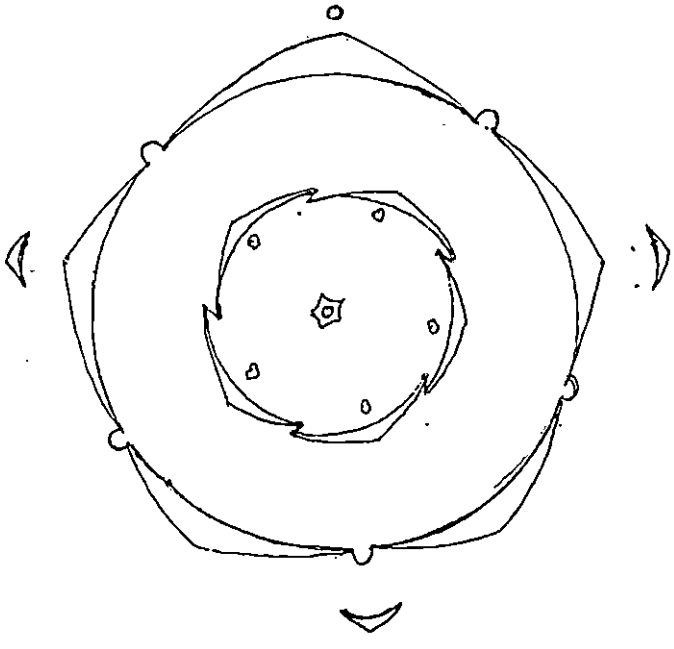
L S OF FLOWER



STAMEN



C.S OF OVARY

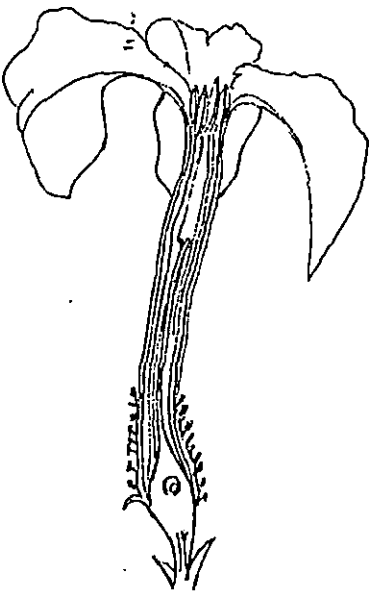


FLORAL DIAGRAM

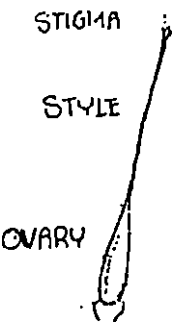
$$\sigma \oplus K_{(5)} C_{(5)} A_5 \underline{G}_1$$

FLORAL FORMULA

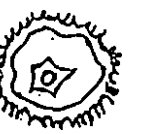
Fig.2 Floral morphology of *Plumbago zeylanica*



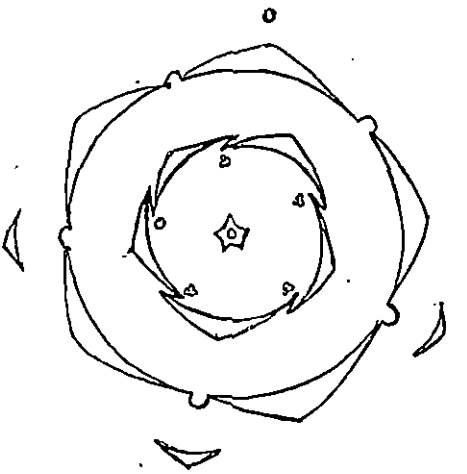
L.S OF FLOWER



STAMEN



C.S OF OVARY



FLORAL DIAGRAM

$$\female \oplus K_{(5)}\, C_{(5)}\, A_5\, \underline{G}_1$$

FLORAL FORMULA

Fig.3 Floral morphology of *Plumbago capensis*

4.2 Anatomical characterization

4.2.1 Stem anatomy

Transverse section from the first internode of the first unflowered primary branch was examined under microscope. Observations on various anatomical characters are presented in Table 4 and plates 9 to 11.

Table 4. Anatomical features of the stem of different *Plumbago spp.*

Characters	*P. rosea*	*P. zeylanica*	*P. capensis*
I. Outline of T.S.	Slightly wavy	Wavy with deep constrictions	Angular
II. Epidermis			
i) Number of layers	One	One	One
ii) Mean size of epidermal cell (x 400)	28.20 µm x 31.20 µm	22.80 µm x 25.20 µm	18.60 µm x 15.60 µm
iii) Epidermal appendages			
Glandular hairs	Absent	Short stalked multicelled globular glands	Short stalked multicelled globular glands
Mean size of glandular hairs (x 400)	-	54.70 µm x 49.60 µm	39.00 µm x 31.80 µm
Non glandular hair	Absent	Absent	Absent
III. Cortex			
Collenchyma	Present	Present	Present
Chlorenchyma	Seen as round patches	Seen as discontinuous ring	Seen as discontinuous ring
Endodermis	Ill-defined	Ill-defined	Ill-defined

IV Stele			
Pericycle	III-defined	III-defined	III-defined
Cambium	3-4 layers	3-4 layers	3-4 layers
Vascular type	Endarch	Endarch	Endarch
Number of bundles	10	10	10
Crystals	Absent	Absent	Absent
Mean size of xylem vessel (μm) (x 400)	41.76 x 50.24	50.40 x 47.50	39.12 x 38.24

From Table 4 it is clear that variations exist among the three species of *Plumbago* with respect to the outline of section, size of epidermal cells, glandular hairs of and the arrangement of chlorenchyma in the cortical region.

4.2.2 Root anatomy

Details of the anatomical characters of the mature roots of the three species of *Plumbago* are presented in Table 5 and plates 12 to 14.

Table 5. Anatomical features of the roots of three different *Plumbago spp.*

Characters	*P. rosea*	*P. zeylanica*	*P. capensis*
Outline of T.S.	Circular	Circular	Circular
Mean thickness of cork (μm) (x400)	79.20	89.00	88.00
Phellem			
Number of layers of cell	1-2	2-3	2-3
Shape of cell	Widely oblong	Narrowly oblong	Oblong
Phellogen			
Number of layers of cells	1-2	2	3
Shape of cells	Oblong	Widely oblong	Oblong

Phelloderm			
Number of layers of cells	2	4	2
Shape of cells	Oblong	Narrowly oblong	Oblong
Cortex			
Mean thickness of cortex (μm)(x400)	1152	328	272
Starch grains	Absent	Abundant	Absent
Stone cells	Absent	Present	Present
Arrangement of stone cells	-	Scattered	Ring like manner
Mean size of stone cells (μm) (x400)	-	18 x 48	24 x 30
Number of layers of phloem cells	3-4	5	6-7
Medullary rays	Clear single row of parenchymatous cells	Clear Two rows of parenchymatous cells filled with starch grains	Clear Single row of parenchymatous cells

From Table 5 it is evident that the roots of the three different species differed with respect to the features of cork, cortex and medullary rays. Starch grains were present in the cortex of the roots of *Plumbago zeylanica*. Stone cells were observed in the sections of *Plumbago zeylanica* and *Plumbago capensis*.

4.2.2.1 Xylem vessel character

Features of xylem of all the three different species of *Plumbago* were studied by macerating internodal segments in Jeffery's fluid. Results of this study are given in Table 6.

Table 6. Xylem vessel characters of three species of *Plumbago*

Character	*P. rosea*	*P. zeylanica*	*P. capensis*
Vessel type	Spiral and Scalariform	Spiral and Scalariform	Spiral and Scalariform
Mean xylem vessels / unit area (mm^2)	59.00	91.00	180.00
Scalariform vessels			
Mean length (μm) (x100)	220.00	144.00	180.00
Mean breadth (μm) (x100)	32.00	32.00	32.00
L/B ratio	6.88	4.50	5.63
Spiral vessels			
Mean length(μm)(x100)	256.00	176.00	200.00
Mean breadth (μm)(x100)	16.00	16.00	16.00
L/B ratio	16.00	11.00	12.50

From the above table it can be seen that the three species of *Plumbago* differed with respect to the length and breadth of xylem vessels even though the type of vessels were the same. The length and breadth measurements were the least in *P. zeylanica.*

4.3 Reproductive Biology

4.3.1 Anthesis time and peak period of anthesis in an inflorescence

A preliminary study revealed that there was no flower opening between 4 p.m. and 2 a.m. Hence bihourly observations on flower opening were taken from 2 a.m. to 4 p.m. The percentage of flower opening at periodic intervals are presented in Table 7 and Fig.4.

Table 7. Anthesis time in different species of *Plumbago*

Species	Total number of flowers observed	Percent of flowers opened						
		2 a.m. to 4 a.m.	4 a.m. to 6 a.m.	6 a.m. to 8 a.m.	8 a.m. to 10 a.m.	10 a.m. to 12 noon	12 noon to 2 p.m.	2 p.m. to 4 p.m.
P. rosea	103	-	-	86	13	-	1	-
P. zeylanica	87	2	48	39	4	5	2	-
P. capensis	64	-	6	58	13	9	9	5

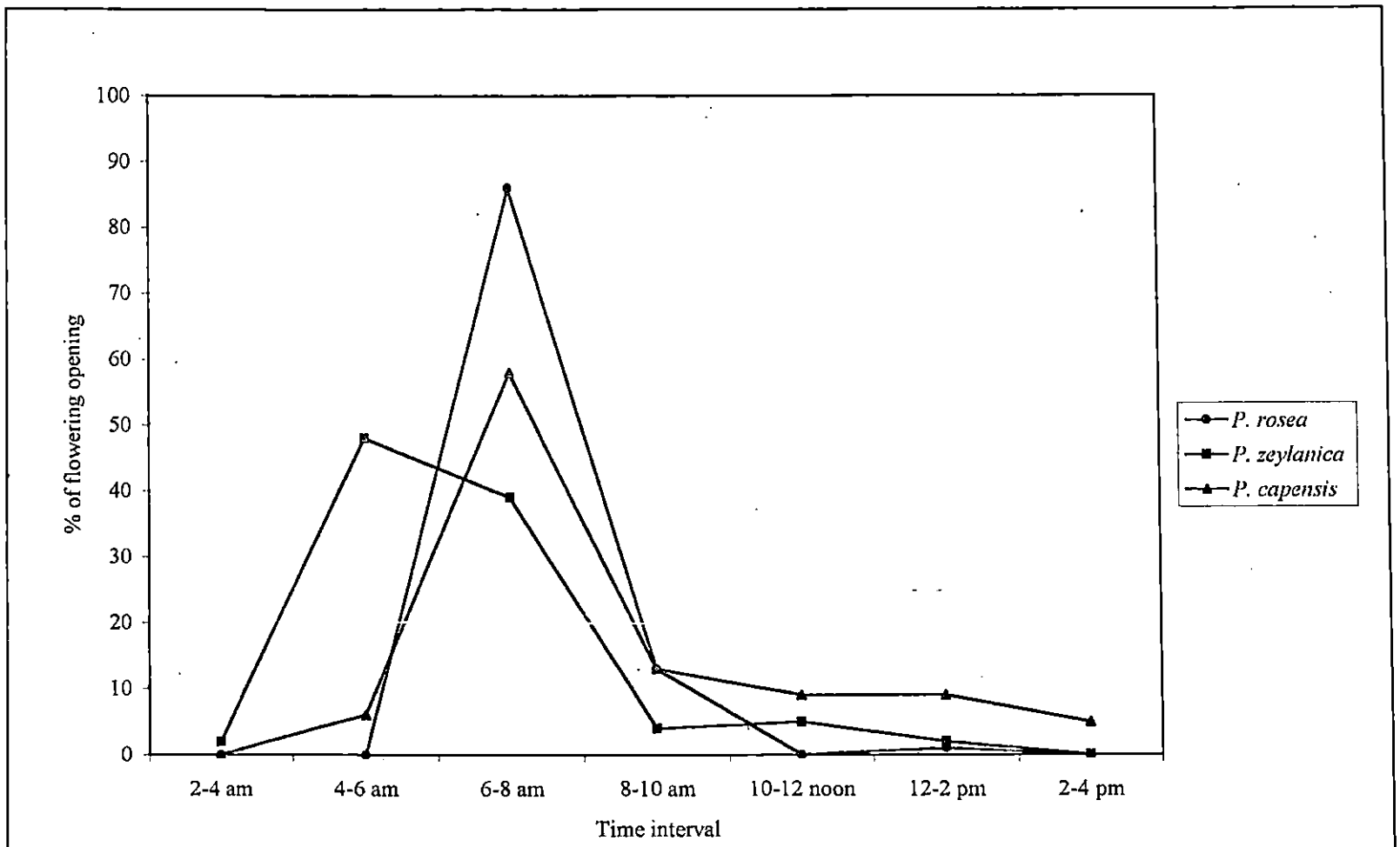


Fig. 4 Proportion of flower opening in different species of *Plumbago*

4.3.2 Time of anther dehiscence and stigma receptivity

Fully mature flower buds were observed for anther dehiscence and stigma receptivity at periodic intervals and the results are presented in Table 8.

Table 8. Time of stigma receptivity and anther dehiscence of different *Plumbago spp.*

Species	Time of anther dehiscence	Starting time of stigma receptivity	Duration of stigma receptivity (minutes)
P. rosea	3 hours after flower opening	1 hour after flower opening	30
P. zeylanica	Coincides with flower opening	Coincides with flower opening	35
P. capensis	2 hours after flower opening	30 minutes before flower opening	25

From Table 8 it is clear that only in *Plumbago zeylanica* the anther dehiscence coincides with stigma receptivity. Observations on insects visiting flowers were taken. *Apis spp.* were found to be frequent visitors. It was also observed that the pollen grains were easily carried off by wind soon after anthesis in all the three species studied.

4.3.3 Pollen morphology and fertility

4.3.3.1 Pollen morphology

The morphology of pollen (plates 15 to 17) collected from fully mature buds were studied in all the three species of *Plumbago* using acetocarmine staining technique, which revealed the following results. The results are given in the Table 9 and Fig.5.

Table 9. Morphological features of pollen in different species of *Plumbago*.

Species	Colour as appeared to naked eye	Type	Mean size (μm) (x100)	Range (μm) (x 100)	Shape	Sculpturing of exine
P.rosea	Creamy white	Tricolpate	54.40	48-64	Spherical	Reticulate
P.zeylanica	White	Tricolpate	80.00	64-96	Spherical	Reticulate
P.capensis	Creamy yellow	Tricolpate	80.00	64-96	Spherical	Reticulate

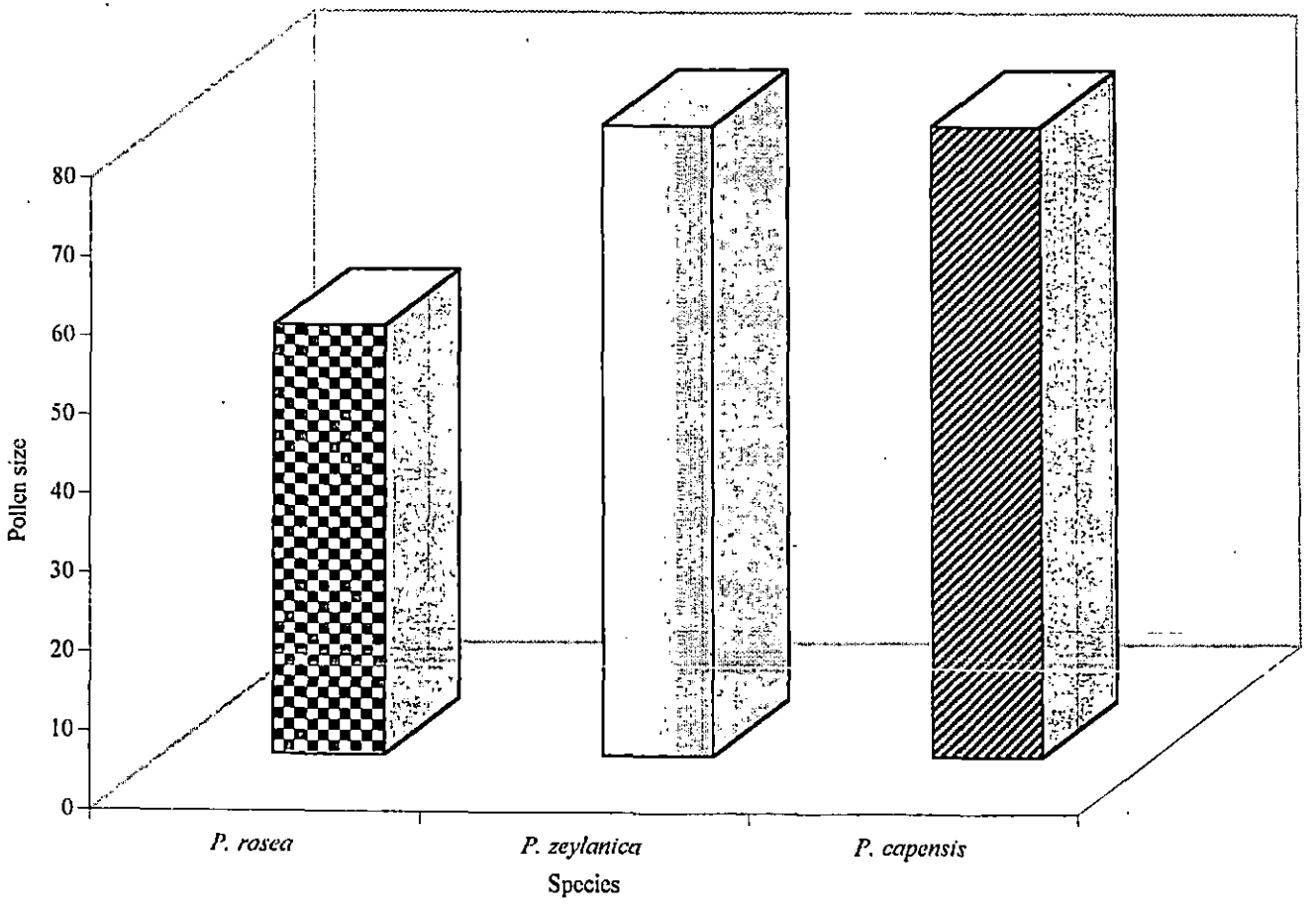


Fig.5 Pollen size in different species of *Plumbago*

The pollen grains were spherical, tricolpate and with reticulate exine in all the three species. Pollen size was the least in *P. rosea* (54.40 µm). The colour of pollen grains also varied with the species.

4.3.3.2 Pollen fertility percentage

The fertility of pollen collected from fully mature buds was assessed by acetocarmine staining which revealed the following results. Fertility % of pollen in different species of *plumbago* are given in Table 10 and Fig.6.

Table 10. Pollen fertility percent in different species of *Plumbago*

Species	Mean fertility %
P.rosea	81
P.zeylanica	89
P.capensis	84

4.3.4 Mode of pollination

Percentage of fruit set under selfing and open pollinated condition was studied in all the three species of *Plumbago*. Results are shown in Table 11.

Table 11. Fruit set percent in different species of *Plumbago*.

Species	% of fruit set	
	Selfing	Open pollinated condition
P. rosea	0	0
P. zeylanica	49	71
P. capensis	0	0

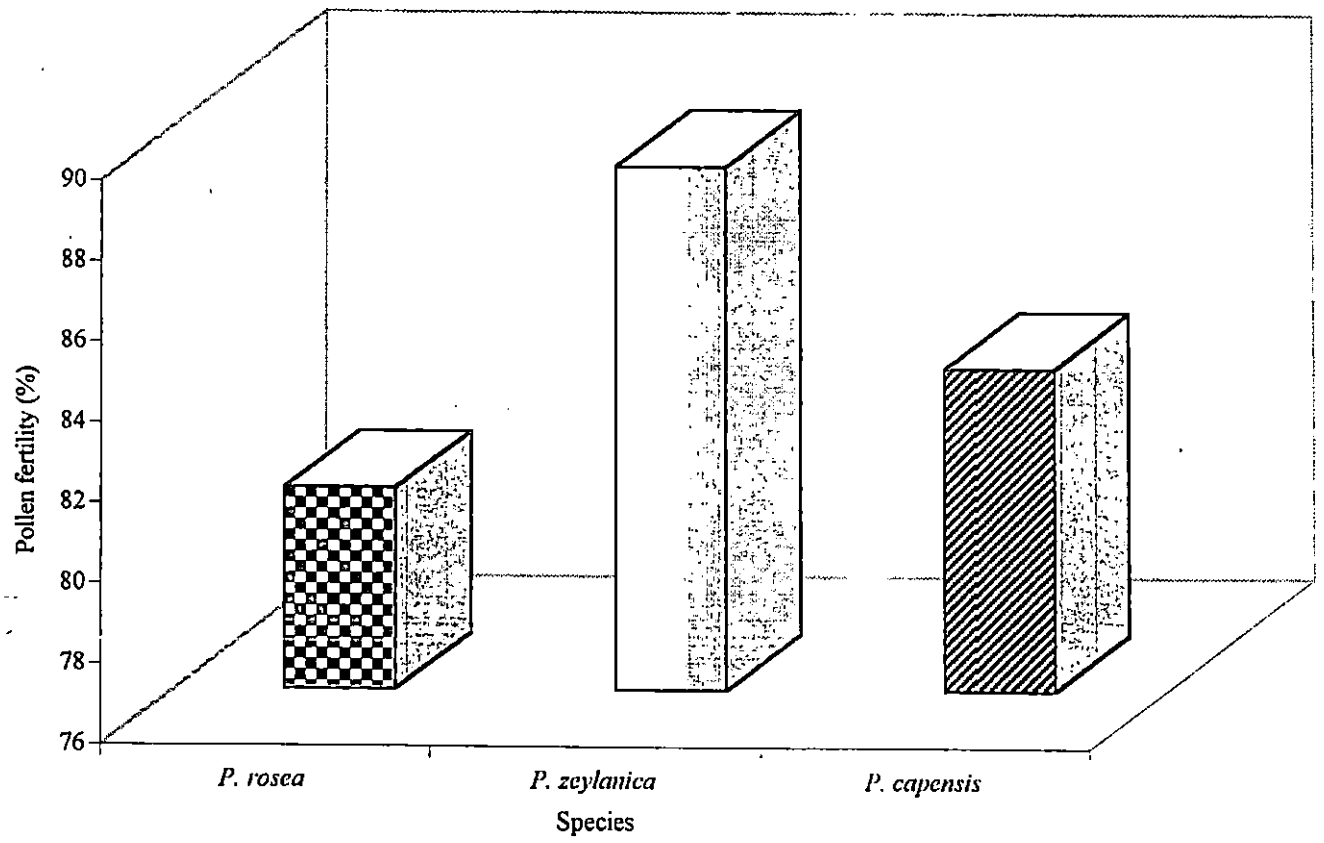


Fig.6 Pollen fertility in different species of *Plumbago*

Among the three species only *P. zeylanica* exhibited fruit set under selfing and open pollinated conditons.

Based on 27 morpho-anatomical and reproductive characters of different *Plumbago spp* listed in Table 12, the pair group method of cluster analysis was done. Overall similarity and dissimilarity matrices were prepared (Table 13a and 13b).

Table 12. Morpho-anatomical and reproductive characters of different species of *Plumbago*

Characters	*P. rosea*	*P. zeylanica*	*P. capensis*	CD (0.05)	C.V. (%)
Mean internodal length (cm)	6.90	6.00	3.10	0.70	15.83
Mean length of inflorescence (cm)	26.63	11.27	4.26	3.55	27.33
Mean number of flower inflorescence	32.40	26.10	12.90	7.06	32.63
Mean distance between flowers (cm)	0.87	0.47	0.32	0.14	27.18
Number of flowers / unit length of inflorescence	1.20	2.26	3.14	7.06	32.63
Mean length of bract (cm)	0.51	0.68	0.84	0.03	6.52
Mean breadth of bract (cm)	0.27	0.29	0.19	0.03	8.46
L X B of bract (cm^2)	0.14	0.18	0.16	0.03	11.39

Mean length of bracteole (cm)	0.39	0.30	0.49	0.03	6.82
Mean breadth of bracteole (cm)	0.19	0.15	0.18	0.03	18.51
L X B of bracteole (cm^2)	0.07	0.04	0.09	NS	19.90
Mean size of calyx tube (cm)	0.85	1.07	1.15	0.06	6.59
Mean size of corolla tube (cm)	2.80	2.30	3.50	0.17	6.30
Mean size of limb (cm)	1.70	0.68	1.20	0.11	11.81
Mean length of stamen (cm)	3.06	2.32	3.50	0.11	4.29
Mean length of style(cm)	2.30	2.27	2.72	0.17	7.40
Mean length of epidermal cell of stem (µm) (x400)	28.20	22.80	18.60	3.07	14.60
Mean breadth of epidermal cell of stem (µm) (x400)	31.20	25.20	15.60	3.24	14.91
L x B of epidermal cell (μm^2) (x400)	841.00	569.37	303.17	101.05	19.13
Mean thickness of cork (µm) (x400)	79.20	89.00	88.00	15.03	19.40
Mean thickness of cortex of root (µm) (x400)	1152.00	328.00	272.0	74.62	14.21
Mean xylem vessels / unit area (mm^2)	59.00	91.00	180.00	4.91	4.91

Mean length of xylem vessel (μm) (x100)	186.00	96.00	153.00	30.00	17.66
Mean breadth of xylem vessel (μm) (x100)	32.00	16.00	32.00	NS	0.47
L/B ratio of xylem vessel (μm) (x100)	5.63	5.97	4.77	0.83	15.50
Size of xylem vessel along horizontal direction in c.s (μm) (x100)	41.76	50.40	39.12	18.60	46.11
Size of xylem vessel along longitudinal direction in c.s (μm) (x100)	50.24	47.50	38.24	22.84	54.03
Mean size of pollen (μm) (x100)	54.40	80.00	80.00	6.42	9.85

Table 13a. Dissimilarity / distance matrix

Species	*P.rosea*	*P.zeylanica*	*P.capensis*
P.rosea	0	6.597	7.931
P.zeylanica	6.597	0	7.044
P.capensis	7.931	7.044	0

Table 13b. Similarity / correlation matrix

Species	*P.rosea*	*P.zeylanica*	*P.capensis*
P.rosea	1	-0.277	-0.558
P.zeylanica	-0.227	1	-0.352
P.capensis	-0.558	-0.352	1

Discussion

DISCUSSION

5.1 Morphological evaluation

The morphological characters of the three species of *Plumbago* viz. *P. rosea, P. zeylanica* and *P. capensis* were evaluated. The results revealed that all the three species showed the general characters of Plumbaginaceae. However, the three species differed in many morphological features.

5.1.1 Vegetative characters

The study of root, stem and leaf characters revealed variations in stem type, mean internodal length, leaf color, leaf attachment and leaf shape (Table 1) among the three species of *Plumbago*. *Plumbago rosea* is having smooth cylinderical stem, *Plumbago zeylanica* ridged stem and *Plumbago capensis* angular stem. Among the three species, the highest mean internodal length was exhibited by *P. rosea* (6.90 cm) and the lowest by *P. capensis* (3.10 cm). Reddish striations were observed in the internodal region of *P. zeylanica*. All the three species though had simple, alternate leaves, reddish tinge was observed on the midrib and petiole of *P. rosea* leaves. *P. capensis* was found to possess characteristic spatulate leaves. *P. rosea* and *P. zeylanica* were having ovate leaves. CSIR (1969) has also reported spatulate leaves and pale blue flowers for *P. capensis*.

5.1.2 Inflorescence characters

Inflorescence type is a spike in all the three species. It is borne either in terminal or axillary position in *P. zeylanica* and *P. capensis*, but was observed only in terminal position in *P. rosea* (Table 2)

A comparison of the inflorescence length of three species showed that it is the shortest in *P. capensis* (4.26 cm). It can also be seen that this comes to only about 1/6 of the inflorescence of *P. rosea* and about 1/3 of that of *P. zeylanica.* However it had the highest number of flowers per unit length of inflorescence. Among the three species it can be seen that there is a gradual reduction in the mean distance between successive flowers in an inflorescence from *P. rosea* to *P. capensis* (Table 2). This can be attributed to be the reason for the highest mean number of flowers per unit length of inflorescence in *P. capensis* (3.14) and the least in *P. rosea* (1.20).

5.1.3 Floral characters

The study revealed that *P. rosea* is having beautiful pinkish-red flowers, *P. zeylanica* with attractive white flowers and *P. capensis* with brilliant blue flowers (Table 3). Each flower is subtended by a bract and two bracteoles in all the three species. A reddish tinge was also observed in bracts, bracteoles and sepals of *P. rosea.* Lanceolate bracts were observed only in *P. capensis.* The size of bracts, bracteoles, corolla tube, stamen and style was found to be the highest in *P. capensis* followed by *P. rosea* and *P. zeylanica* respectively. The

three species exhibited variations in color of anthers, filaments and pollengrains. Results obtained agree with the findings of Iyer and Kolammal (1969). Bracts were larger than bracteoles in *P. zeylanica*. However, they were almost equal in size in *P. rosea*.

Hence, eventhough these three species showed general characters of Plumbaginaceae, they could be identified by specific morphological characters.

P. rosea could be easily distinguished by the presence of smooth stem, dark green leaves with reddish tinch on the petiole and midrib, red flowers with reddish green bracts and bracteoles, fimbricate style and stamens with pink filaments, red anthers and creamy white pollen grains.

P. zeylanica is characterised by the presence of ridged stem with reddish striations along the ridges starting from the node, white flowers with cordate bracteoles, green calyx tube, white filaments carrying purple anthers and white pollen grains.

P. capensis was found to be quite different from the other two species due to the presence of spatulate leaves, angular stem, compact inflorescence with brilliant blue flowers having lanceolate bracts and bracteoles, blue anthers, blue filaments and creamy yellow pollen grains.

5.2 Anatomical characterization

5.2.1 Stem anatomy

There was uniformity in the fundamental structure of the stem in all the three species studied. However, variations were observed in the outline of T.S., size of epidermal cells and glandular hairs and arrangement of chlorenchyma in the cortical region. Glandular hairs were observed only in *P. zeylanica* and *P. capensis* (Table 4). Cortex was found to be heterogenous with collenchyma, chlorenchyma and parenchyma.

5.2.2 Root anatomy

Ample variability, both qualitative and quantitative, was observed in the anatomical characters of the roots of different species of *Plumbago.* Transverse section was found to be circular in all the three species.

Variability was observed for thickness of the cork, shape of cells in the cork region, thickness of the cortex and the nature of medullary rays between the three species.

Roots form the officinal part in *Plumbago* and *P. rosea* is the accepted source of raw drug in our state. It is usually adulterated with the roots of other species of *Plumbago.* The roots of *P. rosea,* the accepted drug source can be easily identified by the presence of thick parenchymatous cortex, absence of starch grains and stone cells and presence of a single row of parenchymatous medullary rays (Table 5).

The anatomical features for the identification of *P. zeylanica* roots are the presence of starch grains in the cortex and medullary rays, the parenchymatous connections between pith and cortex as well as the smaller stone cells arranged in a scattered manner in the inner cortex (Table 5).

Anatomical markers for distinguishing *P. capensis* include absence of starch grains in the cortex and medullary rays as well as large stone cells (24μm x 30μm) arranged in a ring like manner in the inner cortex.

Iyer and Kolammal (1960) also reported the presence of starch grains in the cortex and medullary rays of *P. zeylanica.* Menon (1999) has reported that presence of sclerenchyma in the cortex is an indication of drought tolerence of the crop.

5.2.2.1 Xylem vessel character

Xylem, which forms an important part of vascular system, has been used for classification of plants based on its physiological and phylogenetic importance. Spiral and scalariform vessels were found in all the three species. *P. rosea* was showing the largest vessel elements followed by *P. capensis* (Table 6 and 13). The breadth of vessel elements were uniform in all the species. The scalariform vessels that are large with numerous perforations are considered the most primitive when compared with other simple vessels (Bailey, 1953).

5.3 Reproductive Biology

5.3.1 Time of flowering and peak period of anthesis in an inflorescence

Study of anthesis conducted at bihourly intervals revealed that there is variation in the peak period of anthesis (Table 7) in different species of *Plumbago.* The peak period of anthesis was between 6 a.m. and 8 a.m. in *P. rosea* and *P. capensis.* However, in the case of *P. zeylanica* it was observed to be between 4 a.m. and 6 a.m.

The flower opening extended over a long period in *P. zeylanica* and *P. capensis.* The anthesis started at 2 a.m. and continued upto 2 p.m. in *P. zeylanica* where as started at 4 a.m. and continued upto 4 p.m. in *P. capensis.* Flower opening started much later in *P. rosea* with red flowers i.e. at 6 a.m. and continued for a short period of 4 hours only (Table 7) with peak period between 6 a.m. and 8 a.m.

P. zeylanica and *P. capensis* having light colored flowers, opening started earlier. Among these two species itself, *P. zeylanica* with white flowers, opening started by 2 a.m. and 50 per cent of the flowers opened before 6 a.m.

5.3.2 Time of anther dehiscence and stigma receptivity

Anther dehiscence was found to occur after flower opening in *P. rosea* and *P. capensis* (Table 8). In both these species stigma became receptive before dehiscence of anthers indicating the presence of protogyny. However in *P. zeylanica* anher dehiscence coincided with stigma receptivity.

5.3.3 Pollen morphology and fertility

Species level difference was observed with respect to colour and size of pollen grains. Pollen grains of *P. rosea* were comparatively smaller than the other two species (Table 9).

Very high pollen fertility was observed in all the three species (Table 10) in acetocarmine staining technique. However Arya (1999) has observed lack of pollen germination in different media.

5.3.4 Mode of pollination

Inspite of the high pollen fertility there was lack of seed set in *P. rosea* and *P. capensis* (Table 11). However, it was observed that *P. zeylanica* had 49 per cent fruit set on selfing and 71 per cent under open pollinated conditions. In the case of *P. capensis* stigma became receptive in the bud stage and the receptivity was lost before flower opening itself. Besides, pollen dehiscence occurred only two hours after flower opening. This may be the reason for the lack of seed set under selfing and open pollination in *P. capensis*. Poor seed set under selfing in *P. rosea* can be due to marked protogyny of the flowers. Arya (1999) reported abundant adhesion of pollen grains on the papillate stigma of *P. zeylanica* and seed set under natural condition. Under open pollination also no seed set was obtained in *P. rosea* eventhough stigma became receptive after flower opening. This points to the possibility of the presence of some

incompatibility mechanism also. Arya (1999) has also reported the lack of adhesion of pollen on stigma in *P. rosea*.

In *P. zeylanica* seed set was obtained under selfing (49 %) as well as open pollinated conditions (71 %). In this species stigma receptivity was found to coincide with pollen dehiscence (Table 8). Besides, stamen and style were almost of equal length. Thus simultaneous maturity and close proximity of the essential whorls of the flower in *P. zeylanica* promoted seed set under selfing. Higher seed set under open pollination indicates that it is more adapted to cross pollination. Wind and insects particularly *Apis* spp are found to aid pollination.

The distance matrix (Table 13a) arrived at based on morphological, anatomical and reproductive characters of the three species (Table 12) revealed that the least distance was between *P. rosea* and *P. zeylanica* and the highest between *P. rosea* and *P. capensis.*

This is further justified by the similarity matrix (Table 13 b). The results of the cluster analysis are presented in a phenogram (Fig. 7) which illustrates the relationship of the three species.

Based on the morpho anatomical and reproductive characters of the three species of *Plumbago* a key for identification of different species was also prepared.

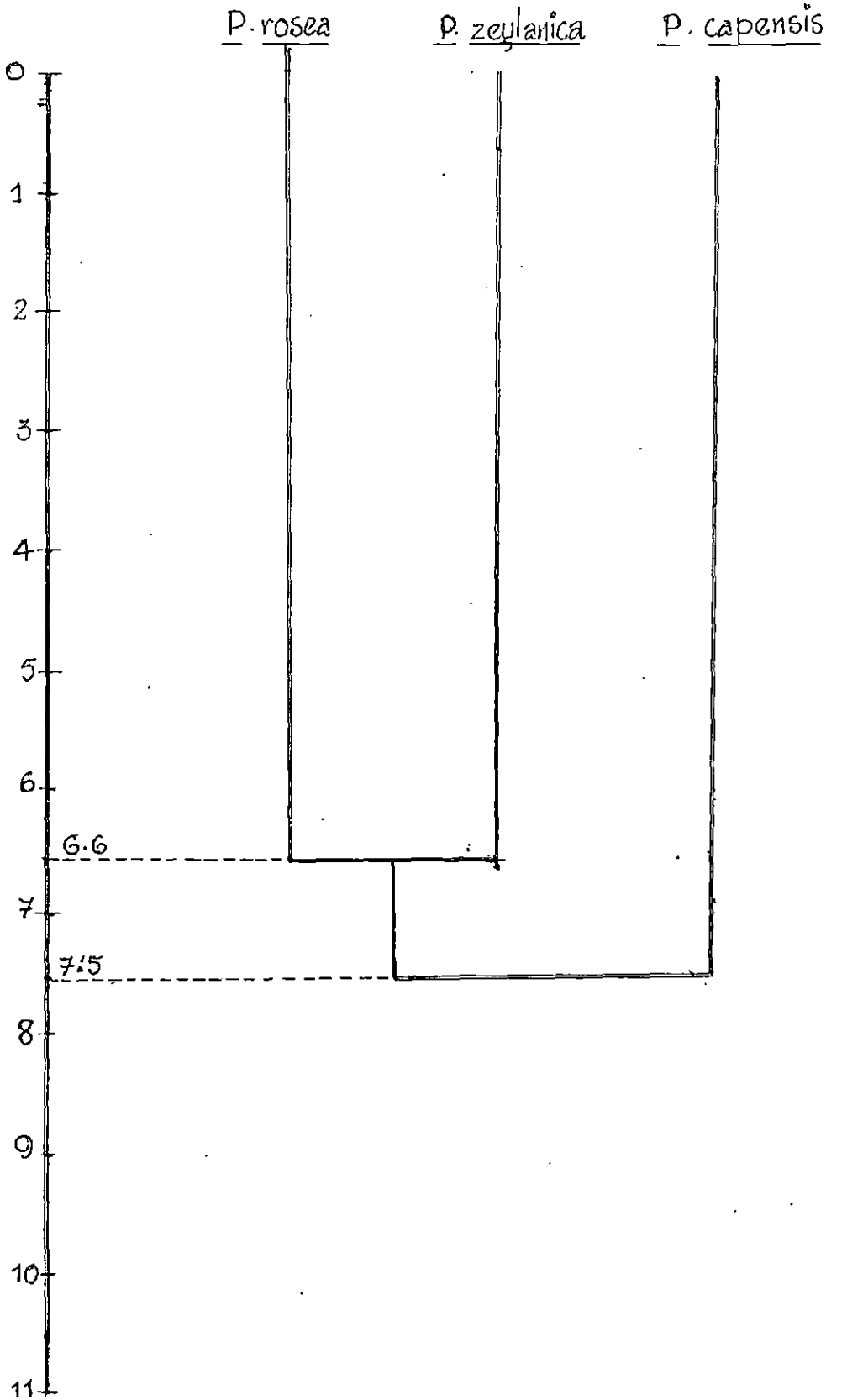


Fig.7 Phenogram of taxonomic relationship of three different species of *Plumbago*

Key for identification of *Plumbago spp.*

1.a Perennial with petiolate ovate leaves, seasonal flowering, flowers with cordate bracts

1. Stem smooth, leaves dark green, petiole and midrib with reddish tinge, long inflorescence with dark green peduncle, flowers red having reddish green bracts, reddish green lanceolate bracteoles, red anthers, pink filaments and creamy white pollen.

T.S. of stem wavy with round patches of chlorenchyma in the cortex Grandular trichomes absent.

T.S. of root with widely oblong phellem cells, absence of starch grains and stone cells in the cortex ----------*P. rosea*

2. Stem ridged with reddish striations starting from the node, leaves green, infloresence short with green peduncle and white flowers having green barcts, green cordate bracteoles, white filaments, purple anthers and white pollen grains.

T.S. of stem wavy with deep constrictions and discontinuous ring of chlorenchyma in the cortex. Grandular trichomes present.

T.S. of root with narrowly oblong phellem cells. Presence of starch grains and scattered stone cells in the cortex.

----------*P. zeylanica*

1.b Perennial with sessile spatulate leaves, flowering continuous, flowers with lanceolate bracts

Stem angular, leaves light green, inflorescence short with light green peduncle and blue flowers having green bracts and green lanceolate bracteloes, blue anthers, blue filaments and creamy yellow pollen grains

T.S. of stem wavy with small epidermal cells, discontinous ring of chlorenchyma in the cortex and glandular trichomes.

T.S. of root with oblong phellem cells, absence of starch grains in the cortex and presence of stone cells arranged in the form a ring in the cortex. ---------- *P. capensis*

Summary

SUMMARY

An evaluation of reproductive biology and morphoanatomical variations among three different species of *Plumbago* was carried out in the Department of Plant Breeding and Genetics during the year 1997-1999. The three different species of *Plumbago* selected for the study were *P. rosea*, *P. zeylanica* and *P. capensis*. The salient findings of the study are summerised below.

1. Though the different species of *Plumbago* showed the basic characters of Plumbaginaceae, they differed in internodal length, stem type, leaf colour, leaf size and leaf attachment.

2. Flowering was found to be seasonal in *P. rosea* and *P. zeylanica* and round the year in *P. capensis*. The inflorescence was terminal in *P. rosea* where as it was both terminal end axillary in the other two species. Inflorescence was very compact in *P. capensis* and it took the least time for completing anthesis.

3. *P. rosea* had pinkish red, bisexual sessile flowers with reddish green bracts and bracleoles, red anthers and pink coloured filaments. *P. zeylanica* had white bisexual sessile flowers with green bracts and bracteoles, purple anthers and white filaments. *P. capensis* had blue sessile bisexual flowers with green bracts and bracteoles, blue anther and blue filament.

4. Transverse section of the stem revealed a slightly wavy outline in *P. rosea*, deep constriction in *P. zeylanica* and angular in *P. capensis*. Though non glandular hairs were absent in all the three species, glanduleer hairs were noticed for *P. zeylanica* and *P. capensis*. Chlorenchyma was arranged as

patches in *P. rosea* where as it was in the form of broken ring in the other two species.

5. Transverse section of root was circular in all the three species. Phellem, phellogen and phelloderm which varied in shape of cells were noticed in all the species. *P. rosea* roots could be distinguished by the absence of starch grains and stone cells. *P. zeylanica* roots were characterised by the presence of abundant starch grains and scatterly arranged small stone cells. The roots of *P. capensis* were characterised by the presence of stone cells arranged in the form of a ring.

6. Spiral and scalariform types of xylem vessels were present in all the three species. The biggest vessel elements were noticed in *P. rosea* and the smallest in *P. zeylanica.*

7. Studies on reproductive biology revealed that peak period of anthesis in *P. rosea* and *P.capensis* was between six to eight a.m. where as it was between four to six a.m. in *P. zeylanica.* Anther dehiscence was found to coincide with flower opening in *P. zeylanica* where as in the other two species it occurred after flower opening. Stigma became receptive after flower opening in *P. rosea* and before flower openingin *P. capensis.* In *P. zeylanica* stigma became receptive at flower opening.

8. Colour, size and fertility of pollen varied with species. However in all the three species pollen grains were tricolpate. Seed set was obtained only in *P. zeylanica* both under open pollinated and self pollinated conditions. A

higher seed set percent (71%) was observed under open pollinated condition. *Apis spp.* And wind are the main agents of pollination.

9. Based on the various morphological, anatomical and reproductive characters a key for the identification of the three species has been proposed.

10. Distance matrix obtained based on 27 morphoanatomical and reproductive characters revealed a close relationship of *P. rosea* with *P. zeylanica* than with *P. capensis.*

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EVALUATION OF REPRODUCTIVE BIOLOGY AND MORPHO-ANATOMICAL VARIATIONS IN *Plumbago spp.*

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ABSTRACT OF A THESIS
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ABSTRACT

A comparative evaluation of the morphological and anatomical features as well as reproductive biology of the three species of *Plumbago* viz., *P.rosea*, *P. zeylanica* and *P. capensis* was carried out in the Department of Plant Breeding and Genetics during 1997-1999 with a view to find out the relationship existing among the species and to prepare a key for their identification.

The different species of *Plumbago* varied with respect to nature of stem and leaves, colour of petiole, leaf attachment etc. Significant variations were also observed in the length of inflorescence, number of flowers per inflorescence time taken for completion of anthesis per inflorescence and size and colour of floral parts.

Though there was uniformity in the fundamental structure of the stem, variations were observed in the outline of T.S., size of epidermal cells and arrangement of chlorenchyma. In the case of root, variability was observed in thickness of cortex, nature of stone cells, presence of starch grains etc.

Study of reproductive biology revealed that anthesis occurred earlier in *P. zeylanica* compared to other two species. Further the period of anthesis also extended over a wide time range in *P. zeylanica.* Eventhough the pollengrains were tricolpate and spherical in all the three species, differences existed with respect to colour, size and fertility of pollen grains. Coincidence of pollen dehiscence and stigma receptivity was found to lead good seed set in *P. zeylanica. Apis spp.* and wind were observed to be the main pollinating agents.

Based on the different morphological, anatomical and reproductive characters a key for the identification of the different species were proposed.

A distance matrix based on morphological anatomical and reproductive characters of three species revealed that *P. rosea* and *P. zeylanica* are more related than *P. capensis.*